OCT 30 1950
©CIB 269974
©CIB 269974
COPY TO L. OF C. OCT 30 1950

VOLUME 8 NUMBER 10

NUTRITION REVIEWS

THE LIBRARY OF CONGRESS SERIAL RECORD
NOV 1 1950
Copy

October 1950

The Thai Diet 289
Comparative Niacin Metabolism 291
Variations in Nutritional Requirements with the Chemical Environment 293
Restriction of Dietary Protein in the Liver 295
Factors Influencing the Occurrence of Neonatal Tetany 296
Growth-Promoting Effects of Antibiotics in Animals 298
The Lymph as a Pathway for Fat Transport 300
Role of Vitamin B_{12} in Metabolism of Methionine 301
Folacin Requirement of Hens 303
Diet and Hypertension 304
Oxalates in the Prevention of Dental Caries in Syrian Hamsters 307
Adrenocorticotropic Hormone and Gastric Secretory Function 309
Massive Liver Necrosis Induced with Yeast Diets in Rats 310
Vitamin Supplements and Performance Capacity 312
Utilization of Phytin by Chicks 317
Notes 319
"Doca" and Ascorbic Acid in Arthritis 319
A New Pancreatic Hormone 319
Determination of the Mass of Single Cells 320

Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK. N. Y.

COPYRIGHT, 1950, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

Assistant Editor

IRENE M. KINSEY, Harvard University, School of Public Health, 695 Huntington Ave., Boston

Associate Editors

JOSEF Brožek, PH.D., University of Minnesota, Stadium South Tower, Minneapolis
HUGH R. BUTT, M.D., Mayo Clinic, Rochester, Minn.
WILLIAM J. DARBY, M.D., PH.D., School of Medicine, Vanderbilt University, Nashville
CHARLES S. DAVIDSON, M.D., Thorndike Memorial Laboratory, Boston City Hospital
R. W. ENGEL, PH.D., Alabama Polytechnic Institute, Department of Animal Husbandry and Nutrition, Auburn
WENDELL H. GRIFFITH, PH.D., Department of Biochemistry and Nutrition, University of Texas Medical Branch, Galveston
JAMES M. HUNDLEY, M.D., Laboratory of Biochemistry and Nutrition, Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Maryland
ROBERT E. SHANK, M.D., School of Medicine, Washington University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ARTHUR H. SMITH, PH.D., Wayne University College of Medicine, Detroit
ESMOND E. SNELL, PH.D., Department of Biochemistry, University of Wisconsin, Madison
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
GEORGE R. COWGILL, PH.D., Yale University
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
J. MURRAY LUCK, PH.D., Stanford University
JAMES S. McLESTER, M.D., University of Alabama
HENRY C. SHERMAN, PH.D., Columbia University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
RUSSELL M. WILDER, M.D., University of Minnesota
JAMES R. WILSON, M.D., American Medical Association
JOHN B. YOUMANS, M.D., Vanderbilt University

Subscriptions should be sent to The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. Rate $3.00 per year in the United States and Canada; $3.50 in other countries.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved. NUTRICION, a Spanish edition of NUTRITION REVIEWS is published by La Prensa Medica Mexicana, Durango 145, Mexico, D.F. Subscriptions should be sent to this address. Rate $3.00 (U.S. currency) or $15.00 (pesos mexicanos).

ERRATUM

Vol. 8, No. 9 page 266, column 1, 2nd paragraph, line 16:
Finch *should read* Fitch

NUTRITION REVIEWS

VOL. 8 OCTOBER, 1950 No. 10

THE THAI DIET

It is well known that some metabolic diseases are among the major causes of death in this country. The causes of such diseases, though still uncertain, are generally thought to be connected with nutrition. A study of dietary habits of different nations of the world may lend a clue to the solution of this problem. The author's experience of nearly twenty years as a physician in Thailand has led him to believe that a diet high in protein and fat may be, or at least may contribute to, the causation of some metabolic diseases. It has been observed that diabetes mellitus and hypertension are more common among the well-to-do urban populations who indulge in high protein and high fat diets, whereas these diseases are comparatively rare among farmers who subsist mainly on rice diets high in carbohydrate, low in protein and fat. Though the validity of such a belief must be proved by future studies, it may be worth while to examine the dietary habits of the common people of Thailand. It is the author's hope that this paper, with its small contribution to the science of nutrition, may be of some interest to the investigators of this country, especially those concerned with public health.

The Rice Diet. Thailand is one of the leading rice-exporting countries of the world. Its soils are so fertile that no farmer considers using fertilizers for improving the crops. Hundreds of varieties of fruits and vegetables are in abundance all the year round. Fish, cattle, and other animal produce are exported to feed neighboring countries. In spite of this plentiful food supply, malnutrition exists. This is due to the general belief handed down from ancient days, and still prevalent among the less educated class of the population, that rice is the only essential food that the human body needs. Any other kind of foodstuff taken with rice is only an accessory which would make rice more palatable and thereby enable a person to consume more rice. A meal is accordingly spoken of as consisting of a "rice" as the main dish and a "with rice" as an accessory and complementary dish. Desserts, which may be fruits or any kind of sweets, are usually served after rice. Generally people take three meals of rice a day, though some are satisfied with two meals, with snacks at noontime.

There are two main varieties of rice. The ordinary rice, the same kind as is used in this country, is consumed in the central and southern part of Thailand, while the people in the north and northeastern part prefer the other variety which is of larger grain, and on account of its stickiness when cooked is called glutinous or sticky rice. Two methods of cooking rice are in general use. The ordinary rice is washed or rinsed with water once or twice and then boiled in a pot. When the rice is almost done the water is drained off and the pot placed on a slow fire until done. The glutinous rice is usually soaked in water for a period of time and then steamed until well done. In either case the rice used is white or polished rice, except in rural areas where the modern machine mill is not available, it is home-pounded brown rice. Parboiled rice, on account of its unattractive color and disagreeable flavor, is never consumed.

Fish, pork, egg, lard, vegetables, peppers, and spices are used to prepare the "with rice." There are many recipes for the preparations, but the common ones may be grouped under three categories, namely, the Kaeng, the Bhud, and the Nam Prik. The Kaeng is the equivalent of the Indian curry.

This consists chiefly of fish or meat cut in small pieces, vegetables, and a mixture of shrimp paste, hot peppers (chili), and spices. All are boiled together and flavored with coconut extract and fish sauce. The Kaeng or curry is usually pepper hot, though there are certain types that are sour or bland. The Bhud is a mixture of vegetables and meat fried in lard and seasoned with fish sauce, and sometimes hot pepper is also added. The Nam Prik usually consists of three separate dishes. The chief one is a mixture of shrimp paste, hot pepper, lemon juice, garlic, and other spices, ground together in a mortar and made into a gravy-like consistency. The second dish is vegetables and leafy greens (*e.g.*, cucumber, egg plant, cabbage, etc.) either raw or cooked. The third dish may be broiled fish or fried eggs. These three components are eaten at the same time with rice.

Nutritional Value of the Rice Diet. It will be seen, as has already been described, that the rice diet in Thailand is far from being similar to the rice diet prescribed by Kempner for hypertensive patients in this country. The major part of the caloric value of the rice diet is derived from rice. This large amount of rice eaten also provides a substantial quantity of vegetable proteins. As source of animal proteins fish constitutes the major part. This is because fish is abundant and procurable almost everywhere. Eggs, pork, and birds are also frequently consumed. Less beef is eaten because the cattle are preserved for farm work. Of the three major foodstuffs, fat seems to be the least consumed. As sources of fats, lard is used in frying and coconut is used in preparing Kaengs. Butter or oleomargarine is never used. Milk never enters into the diet as this is regarded as food for nursing babies only.

Second to rice in the quantity consumed are vegetables and fruits. These are probably the main sources of calcium and other minerals. The intakes of vitamin A, ascorbic acid, and some of the B-complex vitamins are probably taken care of by these groups of foods. Small fish eaten whole are another source of calcium. Vitamin D is probably not needed in food as the sunshine is almost perpetual throughout the year. Cases of rickets have never been observed.

It might well be mentioned here that in the second rural economic survey headed by Dr. James M. Andrews of Harvard University in 1934, the author was the physician in charge of the medical and health survey in the group. The group did not find a single case of clinically clearcut malnutrition. Underweight individuals and anemic individuals were observed and their condition could almost always be attributed to parasites and tropical diseases such as malaria and dysentery. Beriberi seems to be the only avitaminosis recognized in Thailand and this began after introduction of the modern rice mill processes.

A Dietary Survey. The author conducted a dietary survey among a group of soldiers in March 1948. Food intakes were weighed for three consecutive days and samples were taken for analyses. The number of soldiers studied was 137; all were 22 years of age. The average weight and height were 63.9 kg. and 171 cm., respectively. The results of the analyses showed the average per capita intake to be 3300 calories: 76 per cent came from "rice" and 15 per cent from "with rice." In terms of nutrients, 81 per cent was carbohydrate, 10 per cent fat, and 9 per cent protein. The daily protein intake was calculated to be 76 g., 40 g. of which came from rice.

The energy requirements of these soldiers would be expected to be higher than those of the average Thai because of their greater stature and daily training. Especially interesting is the low protein and fat intake, which amounts to 9 and 10 per cent, respectively, of the total caloric intake. In his study on blood pressure in 1928, the author found the average blood pressure among 100 soldiers to be 106/67 mm. Hg. Whether there exists any correlation between low blood pressure and low protein and low fat diet

would be a problem worth while investigating. This should no doubt be done in various age groups with particular emphasis upon elderly subjects.

The National Research Council (U.S.A.), recommends an intake of 70 g. of protein per day for a man weighing 70 kg. On this basis these soldiers, with a protein intake of 76 g. per day, would have a sufficient amount of protein. But it should be noted that over half of the protein intake in this diet came from rice, and the animal protein was estimated to be less than 30 g. These soldiers were conscripted in April 1947 and up to the time of this study they had remained on such a diet for nearly twelve months. As could be gathered from the medical officer-in-charge they remained healthy throughout the period. It would, therefore, be reasonable to conclude that the protein requirements of these soldiers were met even though the animal source was low. This may well be taken as an example demonstrating the supplementary action of the two types of proteins.

It is rather unfortunate in this study that we have no data about the thiamine content of the diet. This was due to lack of laboratory facilities. As far as can be gathered, there were no cases of beriberi among soldiers on such a rice diet. Whether subclinical cases existed were not known. For the same reason of inadequate laboratory facilities, the various mineral nutrients were not determined. The soldiers were on this diet for nearly a year. They appeared healthy and showed no detectable signs of clinical malnutrition. It may be assumed that such a diet is nutritionally adequate.

It is interesting to note that, with the plentiful supply of foods, the people of Thailand, in so far as caloric intake is concerned, do not overeat. This is evidenced from the fact that obesity is not a problem in this country. It is uncommon to see obese persons, especially in the rural areas. This may be attributed to the dietary habit of consuming bulky low fat meals. The bulkiness of the high carbohydrate, low fat rice diet, together with its comparatively low digestibility value consequent on less processing in comparison to the American diet, seems to give satiety with low caloric values.

The question of longevity in relation to the rice diet cannot be evaluated. The shorter expectation of life in Thailand is complicated by many problems such as personal hygiene, sanitation, medical care, and parasitic and tropical diseases.

KAMDHORN SUVARNAKICH
9 Saengchandr Lane
Bangkapi, Bangkok, Thailand

COMPARATIVE NIACIN METABOLISM

For some time it has been known that the manner in which niacin is metabolized by animals is not alike in all species. W. A. Perlzweig, F. Rosen, and P. B. Pearson (*J. Nutrition* **40,** *453 (1950)*) have undertaken an extensive investigation in which metabolic data were obtained after ingestion of niacinamide, N^1-methylniacinamide, and the 6-pyridone of the latter. The following 9 mammalian species were studied: man, dog, rat, pig, rabbit, guinea pig, calf, goat, and sheep. Some of the data reported with the herbivorous species were taken from previously published results by these workers (J. W. Huff, Pearson, and Perlzweig, *Arch. Biochem.* **9,** *99 (1946);* Pearson, Perlzweig, and Rosen, *Ibid.* **22,** *191 (1949)*).

In man, between 75 and 85 per cent of an oral dose of niacinamide could be accounted for in the urine, 30 to 40 per cent as N^1-methylniacinamide, and 35 to 45 per cent as the 6-pyridone. When N^1-methylniacinamide was given orally, only 30 to 40 per cent was recovered in the urine unchanged or as the 6-pyridone. When it was administered subcutaneously, 91 per cent was

recovered, 58 per cent as N^1-methylniacinamide and 33 per cent as the 6-pyridone. From 88 to 92 per cent of a single dose of the 6-pyridone was recovered unchanged in the urine during a subsequent forty-eight-hour period.

These results confirm observations by a number of investigators that niacin metabolism in man involves methylation. The methylated compound is oxidized to the 6-pyridone. W. E. Knox and W. I. Grossman (*Nutrition Reviews* **5,** *223* (*1947*)) reported that the 6-pyridone was a metabolic product of niacin in man.

In the dog, the mechanism for the oxidation of N^1-methylniacinamide is apparently limited. Perlzweig and associates were able to recover only small amounts of the 6-pyridone in the urine after oral administration of N^1-methylniacinamide. The 6-pyridone could not be recovered after oral dosage with niacinamide. The dog, like man, excreted the orally administered 6-pyridone largely unchanged. The dog appears to differ from man in its inability to oxidize the N^1-methylniacinamide to the 6-pyridone. The authors were unable to explain the divergent results obtained with N^1-methylniacinamide administration in this species. Since the methylated compound is generally agreed to be the primary urinary excretory product in the dog, after niacin administration, it was expected that this compound would be largely recoverable unchanged in the urine. However, only 20 per cent of orally administered N^1-methylniacinamide appeared unchanged in the urine. When administered subcutaneously, N^1-methylniacinamide recovery ranged from 85 per cent in one dog to only 2 per cent in another dog. It was suspected that the N^1-methylniacinamide might have been hydrolyzed to trigonelline, but attempts to establish the presence of trigonelline in the urine after N^1-methylniacinamide administration were unsuccessful. It should be mentioned that there is general agreement that trigonelline is not an end product of niacin metabolism in man (*Nutrition Reviews* **5,** *234* (*1947*)).

In the rat, 40 to 50 per cent of orally administered niacinamide, or its N^1-methyl derivative, was excreted as N^1-methylniacinamide and only 3 per cent of these compounds could be recovered in the urine as the 6-pyridone. The rat also excreted the 6-pyridone largely unchanged.

The rat apparently metabolized N^1-methylniacinamide via a pathway not involving the 6-pyridone. In another rodent, the mouse, L. J. Roth, E. Leifer, J. R. Hogness, and W. H. Langham (*Nutrition Reviews* **7,** *166* (*1949*)) observed that niacin or one of its metabolites is decarboxylated. These workers found that about 15 per cent of orally administered niacin, labeled with C^{14} in the carboxyl group, could be accounted for as respiratory carbon dioxide.

The pig excreted both N^1-methylniacinamide and the 6-pyridone after niacinamide ingestion. However, the combined excretions of these derivatives accounted for less than 20 per cent of the niacinamide administered. After N^1-methylniacinamide administration, 3 to 15 per cent appeared in the urine unchanged and 20 to 32 per cent appeared as the 6-pyridone. When the 6-pyridone was administered orally, only 55 per cent was recovered in the urine, indicating that the pig is capable of further metabolizing this compound.

The rabbit excreted less than 4 per cent of orally administered niacinamide as N^1-methylniacinamide or the 6-pyridone, indicating that this species does not possess any great capacity for methylating niacinamide.

In this connection it should be mentioned that P. Handler (*J. Biol. Chem.* **154,** *203* (*1944*)) reported several years ago that rabbits and guinea pigs are not depleted of their "labile methyl" stores by the feeding of niacinamide.

When N^1-methylniacinamide was administered to the rabbit, 17 per cent was excreted unchanged and 25 per cent was excreted as

the 6-pyridone. The enzyme capable of oxidizing N^1-methylniacinamide to the 6-pyridone was demonstrated in rabbit liver by W. E. Knox (*J. Biol. Chem.* **163,** *699 (1946)*). The rabbit excreted the orally administered 6-pyridone largely unchanged.

The guinea pig, like the rabbit, excreted only small quantities of orally administered niacinamide as N^1-methylniacinamide or the 6-pyridone. When N^1-methylniacinamide was administered, the guinea pig, unlike the rabbit, excreted only small quantities of N^1-methylniacinamide (0.5 per cent) or the 6-pyridone (4 per cent).

In the goat, sheep, and the calf, N^1-methylniacinamide excretion ranged from 1 to 12 per cent and the 6-pyridone excretion ranged from 3 to 4 per cent of orally administered N^1-methylniacinamide.

Administration of the 6-pyridone to the guinea pig, calf, goat, and sheep resulted in recoveries of from 10 to 60 per cent, indicating that these species are capable of further metabolizing this compound.

In the calf, sheep, and goat, ingestion of niacinamide resulted in excretion of only 0.5 per cent of the dose as N^1-methylniacinamide and only traces of a substance whose identity with the 6-pyridone was not established.

The herbivorous species excreted relatively large quantities of ingested niacinamide as nicotinuric acid, ranging from 8 per cent of the dose ingested in the guinea pig to 43 per cent in the rabbit.

These results can be briefly summarized as follows: Of the 9 mammalian species studied, only man was capable of oxidizing N^1-methylniacinamide to the 6-pyridone to any appreciable extent. The dog, rat, pig, and rabbit, and to a lesser extent the guinea pig, possess only limited capacities for converting N^1-methylniacinamide to the 6-pyridone and whether the goat, sheep, and the calf form the 6-pyridone is questionable. The results suggest that herbivorous animals metabolize niacin via a pathway that does not involve methylation.

VARIATIONS IN NUTRITIONAL REQUIREMENTS WITH THE CHEMICAL ENVIRONMENT

As the nutritional requirements of living organisms become known in greater detail, it becomes more and more obvious that the magnitude of the requirement of an organism for a given nutrient is not a constant, but may vary greatly depending upon the amounts and variety of other nutrients available to the organism. Many striking instances of such variation have been observed among lower organisms, particularly those having complex nutritive requirements, such as the lactic acid bacteria. A recently discovered example lies in the nutritional equivalence of vitamin B_{12} or of purine and pyrimidine desoxyribosides for *Lactobacillus leichmannii* and several other bacteria. Either the vitamin or one of the desoxyribosides, but not both, must be supplied to permit growth (*cf.* V. Kocher and D. Schindler, *Internat. Ztschr. f. Vitaminforsch.* **20,** *441 (1949)*, E. Kitay, W. S. McNutt, and E. E. Snell, *J. Biol. Chem.* **177,** *993 (1949)*). Additional examples of this phenomenon have been discussed by Snell (*Wallerstein Lab. Communications* **11,** *33 (1948)*).

In each of the cases referred to above, growth has been the criterion of nutritional adequacy. It has also been shown, however, that amounts of a nutrient sufficient to permit maximal growth may not suffice to permit production of cells with a maximal ability to carry out certain enzymatic reactions (*Nutrition Reviews* **5,** *154 (1947)*). For example, the amounts of vitamin B_6 required by *Streptococcus faecalis* for production of maximal amounts of tyrosine decarboxylase

are much larger than those required for maximal growth. Thus the amount of a nutrient deemed adequate to meet the needs of an organism may vary not only with other components of the diet, but also with the particular criterion chosen by the investigator as indicative of an adequate intake.

By extension, one might suspect that substances that are not nutritionally essential in the ordinary sense (*i.e.*, for growth) might be required as nutrients to permit production of cells showing a given enzymatic activity. The validity of this idea was proved in 1948 by D. J. O'Kane and I. C. Gunsalus (*J. Bact.* **56,** *499* (*1948*)). These workers showed that when *S. faecalis* was grown in a medium containing yeast extract, cell suspensions oxidized pyruvic acid rapidly, as indicated by a rapid oxygen uptake in its presence. When the same organism was grown in a nutritionally adequate synthetic medium, cells failed to oxidize pyruvate to acetic acid and carbon dioxide, but did so if yeast extract was added to the cell suspension. Thus an unidentified factor in yeast extract which was not essential for growth under the conditions used, was essential for elaboration of the enzyme system that oxidizes pyruvic acid. This substance, termed the pyruvate oxidation factor, was shown not to be identical with any of the synthetic vitamins or growth factors, and was not required for oxidation of glucose by the organism.

The question may be asked whether presence of the pyruvate oxidation factor, required for elaboration of the system that oxidizes pyruvic acid but not required for growth, confers any advantage upon cells which contain it. A partial answer in the affirmative seems possible on the basis of present data. It has long been known that growth of many lactic bacteria is stimulated by the presence of acetate, and this substance has been added routinely to most defined mediums used for culture of these bacteria. B. M. Guirard, E. E. Snell, and R. J. Williams (*Arch. Biochem.* **9,** *381* (*1946*)) had shown, however, that yeast extract contained a water-soluble factor which duplicated the growth-promoting effects of acetate for *Lactobacillus casei*, but was much more active than acetate on a weight basis. On the latter grounds, it appeared possible that the yeast factor functioned catalytically in permitting formation of acetate by *L. casei*, and this possibility suggested to Snell and H. P. Broquist (*Ibid.* **23,** *326* (*1949*)) that this growth factor might be identical with the pyruvate oxidation factor of O'Kane and Gunsalus. Similarities in properties also led them to suspect a relationship to protogen, an essential growth factor of unknown chemical nature required by *Tetrahymena geleii* for growth (*Nutrition Reviews* **7,** *207* (*1949*)). Concentrates of the latter two factors were then tested and proved to be highly active (up to 130,000 times as active by weight as sodium acetate) in duplicating the growth-promoting effects of acetate for *L. casei*.

Snell and Broquist (*loc. cit.*) cite unpublished data to show that the concentrates also stimulate growth of *S. faecalis* when the latter organism is grown in acetate-free mediums. The results thus indicate the probable identity of the growth factor of yeast which replaces acetate for *L. casei*, the pyruvate oxidation factor, and an unidentified growth essential for *Tetrahymena geleii*. They also show that presence of this factor confers a growth advantage on *L. casei* and *S. faecalis* when acetate is absent, *i.e.*, under conditions where this important biochemical intermediate must be synthesized, but not when acetate is present. Thus the metabolic function of the unidentified substance, as indicated by *in vitro* tests with resting cells, correlates exactly in these organisms with its effects on growth. The work provides an additional informative example of how differences in the composition of the growth medium may be reflected in differences in apparent nutritional requirements.

P. J. VanDemark (*J. Bact.* **59,** *533* (*1950*))

has shown that cells of *S. faecalis* grown on a synthetic medium are unable to oxidize glycerol, but do so when an unidentified factor is supplied from any of several natural materials, including yeast extract. Like those factors described above, this substance was not required for growth of the cells under the conditions used. The few recorded properties of the substance (stability to acid and alkali, distribution) are similar to those of protogen, the acetate factor, and the pyruvate oxidation factor, discussed above. However, direct test of a protogen concentrate indicated that it was inactive. Since protogen is known to occur in more than a single form (*Nutrition Reviews* **7,** *207* (*1949*)), all of which may not prove active in reactivating enzymatic systems *in vitro*, a relationship of these factors is, however, not excluded.

To date, no evidence to indicate a role of these substances in animal nutrition has appeared. It seems certain, however, that one or more interesting new substances, intimately involved in oxidative metabolism, and of the nature of a vitamin or coenzyme, have been discovered, and that the substance (or substances) is widely distributed in nature. Availability of such a substance in pure form will permit an assessment of its role, if any, in the nutrition of higher animals. Further information about the chemical nature and metabolic significance of the compounds will, therefore, be of great interest. In the meantime, the obvious implications to animal nutrition of the types of interplay between nutrients exemplified in the cases discussed above, and their bearing on the validity of the most widely used criterion of nutritional adequacy, should receive serious attention.

RESTRICTION OF DIETARY PROTEIN IN THE LIVER

The liver is involved in the metabolism of each of the major foodstuffs; for that reason it has long been a matter of interest to nutritional biochemists to determine the influence upon this organ exerted by severe restriction of one or more of the basic energy-yielding food materials. With particular reference to proteins, it was observed (T. Addis, L. J. Poo, and W. Lew, *J. Biol. Chem.* **115,** *111* (*1936*)) that, in the course of a seven-day fast, the liver of experimental rats lost 40 per cent of its protein, an observation interpreted to mean that this organ may be an important site of storage for readily available protein (*Ibid.* **115,** *117* (*1936*)). Aside from the functional aspects, it becomes of interest to determine the shift of substance from the various intracellular components of the liver cell under nutritional stress, and in a recent report (E. Muntwyler, S. Seifter, and D. M. Harkness, *Ibid.* **184,** *181* (*1950*)) such histochemical alterations have been described following severe restriction of dietary protein.

Young adult female rats (170 to 234 g. body weight) were given an adequate diet composed of purified food substances for seven days; then half the number was changed to a protein-free ration and the remainder continued on the control diet for twenty-one days. At this time the livers were removed, part being used for moisture and fat determinations and a weighed portion of the remainder being homogenized. The homogenate was fractionated by differential centrifugation (W. C. Schneider, *J. Biol. Chem.* **176,** *259* (*1948*)) into four fractions: the nuclear fraction obtained at 200 G. for ten minutes, the mitochondrial fraction secured by centrifuging the supernatant and washings at 8500 G. for ten minutes, the residual cytoplasmic fraction resulting from the next supernatant and washings by centrifuging at 20,000 G. for one hour, and the microsomatic fraction which remained after removal of the residual cytoplasmic fraction. Each of the four fractions was analyzed for total nitrogen, desoxypentosenucleic acid,

pentosenucleic acid, total phosphorus, and riboflavin.

The average body weight of the control rats was 204 g. and that of the protein-poor group was 165 g., the difference representing the summation of a gain in weight of one group and a loss by the other. The liver weights of the former group averaged 6.63 g., whereas those of the latter group averaged 5.22 g. The hepatic water increased slightly, but there was reduction in the fat content after protein restriction for twenty-one days (S. Seifter, D. M. Harkness, L. Rubin, and E. Muntwyler, *J. Biol. Chem.* **176,** *1371 (1948)*). The livers of the experimental rats contained on the average 5.76 mg. less nitrogen per gram of liver than did the controls, the major loss coming from the microsomatic fraction. Using the values from control rats as a basis for comparison, the losses of total nitrogen were from nuclear fraction, 0; mitochondrial fraction, 22.4 per cent; microsomatic fraction, 34.4 per cent; residual cytoplasmic fraction, 13.7 per cent; and whole liver, 19.3 per cent. While the loss in total phosphorus was far smaller than that of nitrogen and of doubtful statistical significance, the major contributor to the loss was again the microsomatic fraction.

With reference to the desoxypentosenucleic acid, the livers of the experimental animals showed a probably significant increase over the controls, the nuclear fraction accounting for practically all of the desoxypentosenucleic acid. The total liver homogenate of both groups of animals contained about the same concentration of pentosenucleic acid but there was a shift away from the microsomatic fraction to the residual cytoplasmic fraction, changes which parallel those in total nitrogen and phosphorus. Although the percentage distribution of riboflavin in the various fractions was much the same in the two groups of rats, the livers of the protein-deficient rats had less of the vitamin per gram of tissue than did the controls.

From a consideration of the various shifts of material in the different structural fractions of the hepatic cells, it appears that the nuclear fraction maintains a chemical constancy despite the restriction of dietary protein. The greatest changes occur in the microsomatic fraction where there is a decrease of pentosenucleic acid, phosphorus, and nitrogen when calculated on the basis of the total amounts of these components in the homogenate. Again, whereas the microsomatic fraction lost about one-third of its original nitrogen during protein starvation, the losses by other fractions indicate that the "labile protein" of the liver cell is not segregated in any single morphologic entity.

FACTORS INFLUENCING THE OCCURRENCE OF NEONATAL TETANY

Neurologic manifestations including muscular rigidity, irritability, convulsions, and lethargy observed in infancy may give evidence of the occurrence of tetany. This syndrome may develop secondary to rickets or to changes in electrolyte patterns produced by vomiting, hyperventilation, and renal disorders. However, it also may occur in the neonatal period without existence of these precipitating factors. In a recent review evidence was cited which indicated that tetany of the newborn was associated with hypocalcemia, probably resulting from relative insufficiency of parathyroid function in early infancy (*Nutrition Reviews* **8,** *57 (1950)*).

L. I. Gardner and associates (*Pediatrics* **5,** *228 (1950)*) report observations evaluating the possible etiologic roles of calcium and phosphorus intake, parathyroid glands, and the kidneys in neonatal tetany. Of 16 infants with this disorder treated at the Massachusetts General Hospital, none was breast fed. All of these cases were in infants who received cow's milk formulas of one type or

another, an observation which is consistent with that of others (H. Bakwin, *Am. J. Dis. Child.* **54,** *1211 (1937)*).

Cow's milk is known to have a higher concentration of phosphorus than does human milk. The comparative content of phosphorus in milk from human beings and in cow's milk formulas was determined, as was the ratio of intake of calcium and phosphorus in infants on different feeding programs. The average concentration of phosphorus in samples of human milk was 8.7 mg. per 100 ml., while a formula containing fresh skimmed cow's milk contained 50 mg. phosphorus per 100 ml. Concentrations of phosphorus in formulas containing evaporated milk or other cow's milk products varied from 50 to 98 mg. per 100 ml. The ratio of calcium to phosphorus was substantially and uniformly lower in cow's milk formulas than in human milk. This ratio was found to average 2.26 in human milk and varied from 1.00 to 1.27 in various cow's milk formulas. Dietary intake of phosphorus was more than five times as great in a newborn infant fed an evaporated milk formula as in one fed on the breast. Moreover, the infant fed cow's milk is shown to ingest more inorganic phosphorus per square meter of body surface per day than the average adult does of total phosphorus per square meter per day. Data are presented to demonstrate that cow's milk contains less calcium and much less magnesium per unit of phosphorus than does breast milk. Furthermore, the higher buffer value of cow's milk is likely to produce enteric alkalosis, inhibiting calcium absorption in those portions of the intestine in which contents are not sufficiently acid to assure solution of calcium.

It has been shown by other workers that the size of the parathyroid glands increases when the ratio of calcium to phosphorus is low in the diets of experimental animals. Gardner and his associates reviewed the diet records of 15 infants included in a study of parathyroid histology by E. Kaplan (*Arch. Path.* **34,** *1042 (1942)*). It was of interest that all of 8 newborn infants fed cow's milk were stated to have "idiopathic hypertrophy," while none of 7 infants fed at the breast had this change in histology of the parathyroid. It is concluded that "idiopathic hypertrophy" results from the relative and absolute excess of phosphorus in the diet of newborn infants given cow's milk.

Daily determinations were made of serum inorganic phosphorus, serum ionized calcium, and total phosphorus intake in 2 normal breast-fed infants and in 3 infants given a cow's milk formula in the neonatal period. After the third day the subjects fed cow's milk had higher concentrations of inorganic phosphorus in serum than did those fed from the breast. Values for ionized calcium were substantially lower in the former group. Concentrations of inorganic phosphorus and ionized calcium in serum were comparable with those found in neonatal tetany, although there were no clinical manifestations of the syndrome.

Studies of phosphorus, calcium, magnesium, and nitrogen metabolism in another normal newborn infant fed a cow's milk formula revealed that soon after the feeding program was started there was loss of large quantities of phosphorus in the urine, an increase in serum inorganic phosphorus to relatively high levels, and a decrease in ionized calcium and total magnesium in serum. After two weeks calcium lactate was added to the formula to increase the ratio of calcium to phosphorus from 1.05 to 4.80, a ratio twice as high as that of human milk. With the addition of calcium there was a decrease in concentration of serum inorganic phosphorus and an increase to normal concentrations of the ionized calcium and total magnesium of serum. Coincident with these changes there was a decrease in the phosphorus excreted in the urine and an increase in fecal phosphorus. Calcium retention was increased, while nitrogen and magnesium retention was decreased.

Renal clearance of phosphorus was evaluated in other normal infants during the neo-

natal period. It was observed that renal clearance of phosphorus in an infant fed a cow's milk formula was double that of infants fed human milk. When a premature infant fed breast milk for a first period was given a formula containing a cow's milk product, the serum inorganic phosphorus rose to high levels within twenty-four hours and the phosphorus excreted in the urine increased fivefold. Continuation of this feeding formula was associated with further marked increase in urinary phosphorus but a decrease in serum inorganic phosphorus. The opinion was stated that these inverse changes in urine and serum phosphorus may have resulted from compensatory hyperplasia of parathyroid tissue. When the infant was returned to a diet of breast milk, a further decrease in serum inorganic phosphorus occurred and there was a diminution in the urinary excretion of phosphorus.

The observed values for the renal clearance of phosphorus in these investigations indicate that the maximum phosphorus clearance is only 10 per cent of the glomerular filtration rate in the neonatal period. This compares with a clearance of phosphorus about 50 per cent of the rate of glomerular filtration in the adult. Insufficient parathyroid function in the newborn infant probably accounts for depressed phosphorus excretion. The results of these investigations and others indicate that limitation in renal excretion of phosphorus plus the feeding of cow's milk with its relatively high content of phosphorus make possible the occurrence of tetany in the newborn infant. Breast milk with a lower content of phosphorus and a higher ratio of calcium to phosphorus is from these points of view wholly physiologic and the favored diet for the neonatal period. However, cow's milk diluted to one-third and containing added carbohydrate, calcium, and vitamins A, D, and C may be satisfactory as a feeding.

Although neonatal tetany is not a common problem in pediatric practice, it is one which may be prevented if cognizance is taken of the conditions which predispose to its occurrence. Various commercial products for infant feeding contain phosphoric acid added during milk processing. This has effected further reduction in the already low calcium:phosphorus ratio of cow's milk. With some products used in infant feeding the addition of phosphoric acid has been discontinued and hence a standard and higher ratio of calcium:phosphorus is obtained.

GROWTH-PROMOTING EFFECTS OF ANTIBIOTICS IN ANIMALS

It is well known that animal proteins contain one or more substances, termed the "animal protein factor" (APF), necessary for growth of animals fed rations based largely upon certain plant proteins, or upon highly purified proteins (*e.g.*, casein) from animal sources. Following isolation of vitamin B_{12}, this vitamin was shown by a variety of workers to possess APF activity in each of the assay methods proposed for the latter substance. It is still a moot point whether vitamin B_{12} and its naturally-occurring derived forms, *e.g.*, vitamin B_{12b} (J. V. Pierce, A. C. Page, E. L. R. Stokstad, and T. H. Jukes, *J. Am. Chem. Soc.* **71,** *2952* (*1949*)) account for all of the APF activity of animal products, but current evidence indicates that this vitamin is certainly the most important source of APF activity present in such materials (*Nutrition Reviews* **7,** *10*, *136*, *167*, *225* (*1949*)).

Because of their present and potential economic importance to livestock feeders, vitamin B_{12} or APF concentrates are now being widely produced and sold as feed supplements. All such commercial concentrates are derived from the residues of various microbial fermentations, sometimes carried

out especially for the purpose of enhancing the vitamin B_{12} content of the product, and sometimes carried out with the dual purpose of producing vitamin B_{12} and some other product, *e.g.*, various antibiotics. In the experimental evaluation of these concentrates as feed supplements, it was observed that APF concentrates made from cultures of *Streptomyces aureofaciens* (the organism used for production of the antibiotic, aureomycin) produced a growth response in chicks greater than could be produced with pure vitamin B_{12} (E. L. R. Stokstad and co-workers, *J. Biol. Chem.* **180,** *647* (*1949*)). Other workers had also observed differences in the growth responses of experimental animals to different APF concentrates that could not be ascribed to differences in the vitamin B_{12} content of such concentrates, as determined by independent methods.

Further investigations of this phenomenon have now appeared. E. L. R. Stokstad and T. H. Jukes (*Proc. Soc. Exp. Biol. Med.* **73,** *523* (*1950*)) have confirmed the pronounced growth-promoting effects for chicks of fermentation residues from *Streptomyces aureofaciens* when these are added to a variety of rations based on plant protein and supplemented with an excess of vitamin B_{12}. Supplements of dried whey or of fish meal produced no such effects. The magnitude of the growth effect can be seen from the following average weights of individual chicks after twenty-five days on the experimental ration (soybean meal base): basal ration plus vitamin B_{12}, 281 g.; basal ration plus vitamin B_{12} plus aureomycin residue, 360 g. Chemical fractionation of the "growth factor" in the aureomycin residue revealed it to have properties similar to those of aureomycin itself. Consequently, pure crystalline aureomycin was tested and found to produce growth responses of the same type, and of nearly the same magnitude, as those produced by the crude aureomycin residues.

This pronounced and surprising result, growth promotion by an antibiotic, suggested that occasional previous reports of growth stimulation by incorporation of streptomycin or succinylsulfathiazole (P. R. Moore *et al.*, *J. Biol. Chem.* **165,** *437* (*1946*)), or various phenylarsonic acid derivatives (N. F. Morehouse and O. J. Mayfield, *J. Parasitol.* **32,** *20* (*1946*); H. R. Bird, A. C. Groschke, and M. Rubin, *J. Nutrition* **37,** *215* (*1949*)) in the ration of experimental animals might have a similar basis. Experimental trial showed that streptomycin, succinylsulfathiazole, and 3-nitro-4-hydroxyphenylarsonic acid each produced a growth stimulation in chicks similar to that given by aureomycin (Stokstad and Jukes, *loc. cit.*), although the amounts of the various drugs necessary to produce the effect varied widely. The magnitude of such effects in previously reported trials was not as great as those now found, probably because marginal levels of vitamin B_{12} or other nutrients limited the growth rate.

Chemically, these various compounds have little in common, and because of this pronounced nonspecificity their common effect on growth can scarcely be ascribed to their direct utilization in growth processes. Rather it is tempting to ascribe their common effect on growth to their common antibacterial effect on the intestinal flora. A possible objection to this interpretation, however, is the observation (Stokstad and Jukes, *loc. cit.*) that aureomycin residues still displayed growth-promoting activity after a sufficient treatment with alkali to destroy the antibacterial activity of the antibiotic for *Staphylococcus aureus*.

That the above observations, made with chicks, may have general validity in animal nutrition is indicated by the result of collaborative work between T. H. Jukes, E. L. R. Stokstad, and R. R. Taylor at Lederle and T. J. Cunha, H. M. Edwards, and G. B. Meadows at the University of Florida (*Arch. Biochem.* **26,** *324* (*1950*)). These workers showed that additions of crystalline aureomycin (100 mg. per kilogram of ration) to a vitamin B_{12}-containing ration produced the same type of extra weight gains in pigs that

had been observed in chicks. Mixtures of crystalline vitamin B_{12} and crystalline aureomycin duplicated the growth-promoting effect of the crude aureomycin residues in this animal. Again, the effect of aureomycin is not specific, for R. W. Luecke, W. N. McMillen, and F. Thorp (*Ibid.* **26,** *326 (1950)*) report in the same journal that with mixtures of pure vitamin B_{12} and streptomycin the growth-promoting effect for pigs of the APF supplement derived from *Streptomyces aureofaciens* fermentation can be duplicated. As illustrative of the magnitude of the effect in pigs, the following figures, representing the average daily gain in pounds over a six-week period (6 animals per group) were cited: basal ration (soybean oil meal as protein) alone, 0.88; basal ration plus vitamin B_{12}, 0.98; basal ration plus vitamin B_{12} plus streptomycin, 1.48; basal ration plus APF supplement (aureomycin residue), 1.43.

These results seem to open an entirely new vista in experimental nutrition. The rates of gain in growth achieved in certain of these cases are higher than those usually attained on what had previously been considered as optimal natural rations, and this on rations consisting chiefly of inexpensive plant proteins supplemented with vitamin B_{12} and one or another antibiotic. Before such findings are reduced to a practical basis, much work needs to be done to determine the effects of long-continued supplementation with antibiotics on metabolic processes other than growth, and on the possibility of residual toxicity in the meat produced, and so forth. It seems unquestioned that the results will have considerable economic impact in the livestock industry. The result is also of great scientific interest, and much activity may be expected in an effort to elucidate the mechanism of the effect and to explore its various implications for nutrition.

THE LYMPH AS A PATHWAY FOR FAT TRANSPORT

B. Bloom and co-workers (*J. Biol. Chem.* **184,** *1(1950)*) have re-examined the quantitative significance of the lymph as a pathway for the transport of absorbed fat. Palmitic acid with C^{14} in the carboxylic group was administered either as the free acid or as tripalmitin and the C^{14} then estimated in the thoracic duct lymph, in intestinal lymph, or in the lipids of the liver.

A modification of a method for collecting lymph from the unanesthetized rat (J. L. Bollman, J. C. Cain, and J. H. Grindlay, *J. Lab. Clin. Med.* **33,** *1349 (1948)*) was employed; a polythene canula was inserted into the thoracic duct under anesthesia and fixed in position with ligatures. Intestinal lymph was similarly obtained from one of the intestinal lymphatic trunks. Withdrawal of lymph samples was not begun until after twenty to fifty-three hours after establishment of the fistulas and while the unanesthetized rats were in a restraining cage. The animals began to drink salt solution within six hours and to eat food within twelve hours of the initial operation.

The labeled fat or fatty acid was administered by intragastric intubation under brief (five minutes) anesthesia. At the end of the period of observation, the rats were killed and the lipids remaining in the gastrointestinal tract as well as those in the feces were removed and the C^{14} contained in the lipids was estimated by counting the aliquot of the petroleum ether extracts placed directly on aluminum discs that had been covered with lens paper (C. Entenman, S. R. Lerner, I. L. Chaikoff, and W. G. Dauben, *Proc. Soc. Exp. Biol. Med.* **70,** *364 (1949)*). Fatty acids prepared from the petroleum ether extract of lymph were likewise examined for C^{14} content.

From 4 rats receiving labeled palmitic acid and from 6 rats given labeled tripalmitin, thoracic lymph was collected for nine-

teen to twenty-four hours, 36 to 118 ml. being collected in all after the isotope-containing lipids were administered. In 4 rats with intestinal lymph trunk fistulas, 24 to 46 ml. were collected in twenty-four hours. In 12 of the 14 animals studied, 81 to 95 per cent of the administered fatty acid-C^{14} was absorbed; in 9 animals from 69.8 to 91.8 per cent of the absorbed palmitic acid-C^{14} was recovered in the thoracic duct lymph, and in 4 rats from 69 to 84 per cent of the absorbed fatty acid-C^{14} was recovered from intestinal lymph.

For generations, beginning students in physiologic chemistry have been taught that some two-thirds of the fat absorbed from the gut is transported in the lymph. This concept rests upon an old observation of a patient with a lymph fistula in the leg (I. Munk and A. Rosenstein, *Virchow's Arch.* **123,** *230* (*1891*)). These workers were able to recover as much as 60 per cent of the fat of an ingested meal in the lymph issuing from the fistula. Later investigators have not recovered such large proportions of administered lipid in the lymph; only some 20 per cent was found in the thoracic duct lymph in twelve hours in anesthetized dogs (H. C. Eckstein, *J. Biol. Chem.* **62,** *743* (*1925*)).

The observations of Bloom *et al.* (*loc. cit.*) have the distinct virtue of having been secured on unanesthetized animals and therefore constitute corroborative evidence, of the first order, from the classic studies of Munk and Rosenstein. However, even the most recent data showing incomplete recovery indicate that there is some other pathway over which a small proportion of absorbed fat enters the systemic circulation. The portal vein immediately comes to mind, though the very small amounts of C^{14}-labeled lipids in the liver of the rats described in the present study might be taken as an argument against portal transport.

ROLE OF VITAMIN B_{12} IN METABOLISM OF METHIONINE

Some observations have indicated that the "animal protein factor" (APF) and vitamin B_{12} play a role in the metabolism of methionine. A. R. Patton, J. P. Marvel, H. G. Petering, and J. Waddell (*J. Nutrition* **31,** *485* (*1946*)) noted that supplementation of a corn-soybean diet with methionine produced an extra growth response in chicks. When sardine meal was added to the ration at a level of 2 per cent, no additional growth response to methionine was obtained even though the sardine meal did not supply sufficient methionine to account for its effect in terms of this supplement.

More recently, vitamin B_{12} has been shown to protect weanling rats against kidney hemorrhage induced by diets low in methionine and choline (A. E. Schaefer, W. D. Salmon, and D. R. Strength, *Proc. Soc. Exp. Biol. Med.* **71,** *193* (*1949*)), and to reduce the choline requirement of young chicks (*Ibid.* **71,** *202* (*1949*)). V. A. Drill and H. M. McCormick (*Ibid.* **72,** *388* (*1949*)) showed that vitamin B_{12} concentrate exerted a lipotropic effect in rats maintained on a high fat diet. Perhaps the most conclusive evidence for such an interrelationship consists in the observation, reported over a year ago but not yet published, that *either* methionine *or* vitamin B_{12} permitted growth of *Escherichia coli* under defined conditions in a medium containing sulfonamides (W. Shive, *Ann. N. Y. Acad. Sci., in press*).

Several short papers that bear on this subject have appeared. P. György and C. S. Rose (*Proc. Soc. Exp. Biol. Med.* **73,** *372* (*1950*)) have studied the effect of vitamin B_{12} on various forms of liver injury in rats. The vitamin was ineffective in reducing the incidence or severity of the massive hepatic necrosis observed in rats fed diets high in yeast. Vitamin B_{12} did, however, show a distinctly lipotropic effect in rats receiving a low protein-low fat ration limited in its

choline-methionine content. Illustrative figures for the fat content of the livers of rats after twenty-one days on the various regimens were: control (low protein-low fat) ration, 35 per cent; control plus vitamin B_{12}, 20.5 per cent; control plus methionine, 14.6 per cent; control plus methionine plus vitamin B_{12}, 10.4 per cent. Corresponding reductions in average weight of the livers of the animals also were observed. It should be noted that methionine (50 mg. per day) was more effective than vitamin B_{12} (0.5 microgram per day) as a lipotropic agent under these conditions; again, however, the two substances show similar effects.

The mechanism by which methionine and vitamin B_{12} produce these common effects has been partially clarified. T. H. Jukes, E. L. R. Stokstad, and H. P. Broquist (*Arch. Biochem.* **25,** *453 (1950)*) obtained chicks from eggs laid by hens on an APF-free diet. These chicks were then fed a ration deficient in both methionine and vitamin B_{12}, variously supplemented with these and related factors. Chicks given vitamin B_{12} alone grew very slowly, but rapid growth was permitted when methionine, homocystine, or homocystine plus betaine were given in addition to vitamin B_{12}. In marked contrast, those chicks not receiving vitamin B_{12} showed a good growth response to methionine, but none whatsoever to homocystine or to homocystine plus betaine. This seems to be a clear indication that vitamin B_{12}-deficient chicks, in contrast to normal chicks, are unable to transform homocystine into methionine, and consequently, that vitamin B_{12} is somehow involved in this transformation.

A similar conclusion was reached in another way by E. L. Oginsky at the Merck Institute for Therapeutic Research (*Arch. Biochem.* **26,** *327 (1950)*). By use of the technics described by J. W. Dubnoff (*Ibid.* **17,** *327 (1948)*) she compared the amounts of methionine formed from homocystine plus choline or betaine in homogenates of livers from rats fed upon vitamin B_{12}-deficient diets, and upon these same diets with supplementary vitamin B_{12}. In every case, methionine formation was suppressed in liver homogenates from vitamin B_{12}-deficient animals as compared to those from vitamin B_{12}-sufficient animals, and this was true whether the methyl donor was choline or betaine. A deficiency in ability to effect transmethylation from betaine to homocystine thus was present in the deficient animals. It was not possible to increase the amount of methionine formed in these homogenates by direct addition of vitamin B_{12}, but it does appear that vitamin B_{12} is involved some way in the formation or the activation of the enzyme system that forms methionine from homocystine.

Jukes, Stokstad, and Broquist (*loc. cit.*) noted that their chicks suffering from a double deficiency in methionine and vitamin B_{12} grew more rapidly when *both* methionine and vitamin B_{12} were supplied than when methionine alone was supplied. From this result it appears certain that the function of vitamin B_{12} in animals is not restricted to activation of methionine formation. It is pertinent to note here, therefore, that much microbiologic evidence indicates that vitamin B_{12} also is necessary for formation of various desoxyribosides, since either the vitamin or any of several desoxyribosides, but not both, are essential for growth of several lactic acid bacteria (*cf.* C. E. Hoffmann, E. L. R. Stokstad, A. L. Franklin, and T. H. Jukes, *J. Biol. Chem.* **176,** *1465 (1948)*, E. Kitay, W. S. McNutt, and E. E. Snell, *Ibid.* **177,** *993 (1949)*). The latter results have been secured uniformly in mediums that contained methionine, and in which, consequently, the vitamin would not be necessary for methionine formation.

The rapid progress in discovery of certain functions of vitamin B_{12} evidenced by these results leads to the hope that the detailed mechanisms by which these results are effected may soon be discovered, and with them a greater insight into the profound effects of this vitamin upon growth.

FOLACIN REQUIREMENT OF HENS

M. L. Sunde and co-workers (*Poultry Sci.* **29,** *220* (*1950*)) established the quantitative requirement for folacin (a synonym for folic acid, adopted by the American Institute of Nutrition, April 1949) by White Leghorn pullets.

The basal ration used had the following percentage composition: sucrose 64, hot alcohol-extracted casein 18, gelatin 5, salts IV 5, soybean oil 3, vitamin A and D feeding oil (400-D-3000 A) 2, and fish solubles 3. Vitamin supplements consisted of thiamine, riboflavin, niacin, pyridoxine, calcium pantothenate, choline chloride, biotin, inositol, vitamins E and K. According to microbiologic assay, the basal ration contained 8 micrograms of folacin per kilogram.

Forty pullets were used in the experiment; 4 in each of nine groups received folacin supplements ranging from 0 to 3.0 mg. per kilogram, and one group, serving as positive controls, received a practical ration. The study was conducted for a period of eleven weeks.

Egg production declined rapidly in the birds receiving no folacin, and this was accompanied by severe body weight loss.

Typical folacin-deficient embryos were observed in the eggs from hens on the deficient diet or those receiving only 0.25 mg. of folacin per kilogram. Among the abnormalities noted were bending of the tibiotarsus, syndactyly, parrot beak, and deformed upper mandible. Pipping of the air cell of the egg with insufficient strength to break the shell at hatching was also a common defect among the deficient embryos.

The folacin content of the egg varied with the level of folacin supplied in the diet, ranging from 18 micrograms per gram of egg yolk in eggs from pullets on the diet without folacin supplement to 375 micrograms per gram of yolk in eggs from pullets receiving 3.0 mg. of folacin per kilogram of diet. The values reported for eggs from pullets on the practical ration ranged from 76 to 162 micrograms per gram of yolk and were about comparable to those found in eggs from pullets receiving the basal ration supplemented with 1 mg. of folacin per kilogram.

The folacin contents of the livers of the pullets which had received the practical ration and those which had received 0.5 mg. of folacin per kilogram on the experimental diet were about equal, 2.4 and 2.5 micrograms per gram, respectively. Somewhat less liver storage of folacin was noted in the pullets which had received the basal diet without folacin supplement (2 micrograms per gram of liver). Dietary folacin supplements of 0.75 to 3.0 micrograms per kilogram of diet resulted in somewhat better liver storage in the pullets (3.2 to 4.7 micrograms per gram of liver).

Values for folacin in the liver ranged from 7.3 to 10.0 micrograms per gram of liver in chicks from pullets on the practical ration. To accomplish comparable storage of folacin in the liver in day-old chicks from hens on the purified diet, a folacin supplement of 1 mg. per kilogram of diet was required.

Finally, further evidence was obtained to indicate a relation between the quantity of folacin in the egg, in the liver of the day-old chick, the quantity of folacin in the hen's diet, and the viability of the chicks. All chicks hatched during the last three weeks of the experiment were placed on a folacin-deficient diet. Only those chicks from hens which had received 1.0 mg. or more of folacin per kilogram of diet survived the test period of three weeks on the folacin-deficient diet.

To summarize, about 0.25 mg. of folacin per kilogram of ration was required for body weight maintenance. The data indicated that slightly more than this amount was needed for satisfactory egg production. Five-tenths milligram of folacin per kilogram of diet was sufficient for good hatchability, but 1.0 mg. per kilogram of diet was needed for

storage of sufficient quantities of this vitamin in the egg to insure good liveability of the offspring.

It should be mentioned that earlier reports have been published dealing with the quantitative needs of folacin by breeding hens (L. W. Taylor, *Poultry Sci.* **26,** *372* (*1947*), B. S. Schweigert, H. L. German, P. B. Pearson, and R. M. Sherwood, *J. Nutrition* **35,** *89* (*1948*)). Taylor found the requirement for breeding hens to be slightly more than 0.12 mg. per kilogram of diet whereas Schweigert and associates found the requirement to be somewhere between 0.12 and 0.42 mg. per kilogram of diet. These workers, however, did not use purified diets. W. W. Cravens and J. G. Halpin (*Ibid.* **37,** *127* (*1949*)) had earlier found that between 0.5 and 1.0 mg. of folacin per kilogram of purified diet was required for normal hatchability. The present study was initiated to establish more accurately the folacin requirements for good hatchability and good liveability of the offspring.

DIET AND HYPERTENSION

The nutritional aspects of hypertension (*Nutrition Reviews* **6,** *293* (*1948*); **7,** *312* (*1949*)) and of arteriosclerosis (*Ibid.* **8,** *41*, *61*, *107* (*1950*)), a disease frequently associated with hypertension, have been considered on several occasions in this journal. An article on hypertension and nutrition by F. M. Allen (*Ibid.* **7,** *257* (*1949*)) was devoted largely to his work and focused on the role of salt and the effects of severe salt restriction.

The literature on the treatment of hypertension by restriction of salt intake was reviewed in historical perspective by C. B. Chapman and T. B. Gibbons (*Medicine* **29,** *29* (*1950*)). The review, with its two hundred and twenty-six references, covers also questions of caloric intake, protein intake, and other dietary factors that have been considered in reference to blood pressure. Adequate studies of the role played by nutrition in the genesis of hypertension in man are not available. Consequently, most of our information is based on the results obtained in the course of treatment of the disease.

Among the attempts to reduce blood pressure by dietary means, the use of salt deprivation is the oldest and most common. Low salt diets, including the milk diet recommended by J. A. Chrestien (*Arch. gén. de méd.* **27,** *329*, *484* (*1831*)) were first used as a therapeutic measure in edematous states. In France, F. Widal (*Semaine méd.* **23,** *219* (*1903*)) and C. Achard (*Ibid.* **23,** *259* (*1903*)) promulgated the idea that chloride retention was the essential pathogenic factor in cardiac and nephritic edema and used restriction of chloride intake as a therapeutic measure for these conditions. L. Ambard and E. Beaujard (*Ibid.* **25,** *133* (*1905*)) applied the principle to renal hypertension, considered as a "dry" chloride retention, *i.e.*, not associated with edema. L. Blum (*Verhandl. d. Kong. f. inn. Med.* **26,** *122* (*1909*)) suggested that the dominant element in water retention in patients on high salt intake is the sodium ion, not the chloride, and experimental support for this thesis was provided by A. Magnus-Levy (*Deutsch. med. Wchnschr.* **46,** *594* (*1920*)), among others. Are, then, the effects of salt restriction on the lowering of blood pressure in hypertensive patients due, in fact, to the effects of the sodium ion? This point has been hotly debated for over twenty-five years. Clinical experience as well as experimental data tend to favor this explanation. A. Grollman and T. R. Harrison (*Proc. Soc. Exp. Biol. Med.* **60,** *52* (*1945*)), studying rats with experimentally-induced hypertension, found that addition of sodium chloride to a low salt diet prevented the hypotensive effects, whereas addition of potassium chloride did not interfere with lowering of blood pressure.

The therapeutic benefits of rigid sodium chloride restriction to patients with hypertension have been advocated, since 1920, by Allen. Out of 104 patients with essential hypertension, three-fourths were reported to have benefited, although in some patients the effect was only temporary (F. M. Allen and J. W. Sherrill, *J. Metab. Res.* **2,** *429 (1922)*). The diet was adequate in calories, and provided 50 to 100 g. of protein and 0.50 to 0.75 g. of sodium chloride daily. In the twenties, several workers were able to confirm the effectiveness of salt deprivation in the treatment of hypertension, although the results were less impressive. In time there appeared dissident voices, denying the benefits, and interest in this method of treatment of hypertension waned.

Interest was revived, in 1944, as a result of the work of W. Kempner (*North Carolina Med. J.* **5,** *125, 273 (1944); 6, 61 (1945)*). Like Allen, Kempner seeks the cause of hypertension in the overworked kidney and the aim of the dietary treatment is to lessen the work load of the kidney by limiting the intake of salt, protein, fat, fluid (and calories). The Kempner diet consists of rice and sugar, fruit, and fruit juices. It provides a daily intake of about 2000 calories, 15 to 25 g. of protein, 4 to 6 g. of fat, no cholesterol, 0.25 to 0.40 g. of chloride, and 0.10 to 0.15 g. of sodium. Fluids are limited to 700 to 1000 ml. a day. When the patient has improved, some nonleguminous vegetables, potatoes, lean meat, or fish may be added to the diet.

In 1948 Kempner reported observations based on 500 patients with "hypertensive vascular disease" (*Am. J. Med.* **4,** *545 (1948)*). A decline of 20 mm. or more in the "mean" blood pressure was observed in 62 per cent of the patients, the systolic blood pressure responding more readily than the diastolic blood pressure. The changes in blood pressure became evident after periods of treatment varying from four days to ten months. In evaluating the results of the diet therapy, Kempner utilized several other criteria, in addition to the changes in blood pressure, such as reduction of the enlargement of the heart and improvement of electrocardiographic abnormalities and of severe pathologic changes of the eye grounds. A substantial decrease in the size of the enlarged heart was reported for 44 per cent of the patients with cardiac enlargement, and reversion to normal of the T wave in lead I of the electrocardiogram in 48 per cent of the patients with a diphasic or inverted T wave. There was a decrease in serum cholesterol and serum chloride, and a fall in the urinary sodium and chloride. There were, also, some decreases in body weight ascribed by Kempner mainly to the loss of "visible or invisible edema."

The effects of salt restriction were tested by several other investigators. The blood pressure was found to be lowered in some hypertensives (*see e.g.*, J. M. Bryant and E. Blecha, *Proc. Soc. Exp. Biol. Med.* **65,** *227 (1947)*; A. W. Contratto and M. B. Rogers, *New Engl. J. Med.* **239,** *531 (1948)*; D. G. Loofbourow, A. L. Galbraith, and R. S. Palmer, *Ibid.* **240,** *910 (1949)*), but the percentages of patients who improved have varied. The variation in the criteria of "improvement" and the frequent absence of control observations make a strict comparison of different studies difficult, if not impossible.

Several features of the Kempner dietary regimen were criticized. In particular, the claim that adult patients receiving the diet will remain in nitrogen balance does not seem to be tenable (W. B. Schwartz and J. K. Merlis, *J. Clin. Invest.* **27,** *406 (1948)*). In view of the importance of both sodium and chloride in the internal environment of the body, the dangers of prolonged, rigorous restriction of salt should not be neglected. The subject of heat cramps, resulting from excessive loss of salt through sweatings in hot environments, coupled with inadequate salt intake, was reviewed by J. H. Talbott (*Medicine* **14,** *323 (1935)*). Early symptoms of salt depletion include dizziness, headache,

anorexia, nausea, apathy, and muscle twitchings.

In the thirties, attention was drawn by H. Strauss (*Fortschr. Therap.* **6,** *70 (1930)*) and by several French workers, to the development of a syndrome, characterized by the rise of urea nitrogen in the blood, which was occasionally found in patients on low salt diets. The risk of uremia associated with sodium depletion was pointed out by W. B. Macguire (*J. Am. Med. Assn.* **137,** *1377 (1948)*). L. A. Soloff and J. Zatuchni (*Ibid.* **139,** *1136 (1949)*) reported that salt depletion may have fatal results. Thirty cases of "low-salt syndrome" were investigated by H. A. Schroeder (*J. Clin. Invest.* **28,** *809 (1949)*). Treatment by means of salt deprivation of hypertensive patients with renal insufficiency was one of the conditions in which the syndrome was encountered. This counsels caution in the use of low salt therapy. On physiologic analysis, a prolonged negative salt balance is undesirable and eventually must lead to serious disturbances in the functioning of the machinery of the body. After the body is freed from excess salt, a salt balance must be maintained by adjusting the salt intake to the amount needed for replacement of the losses.

The effect of reduced caloric intake on blood pressure is difficult to evaluate because the changes in amount are frequently associated with changes in the composition of the diet. This is certainly the case in the conditions of natural starvation and is likely to be true also of therapeutic diets. The fact that weight reduction tends to be accompanied by a lowered blood pressure cannot be doubted. Slight to moderate hypotension in uncomplicated prolonged undernutrition was seen during World War I and was reported again, in experimental as well as natural conditions, during World War II. In normotensive young men who lost about one-fourth of their body weight the mean systolic pressure fell from 106.5 to 94.7 mm.Hg., the mean diastolic pressure decreased from 69.9 to 64.5 mm.Hg. The trend was reversed during refeeding (J. Brožek, C. B. Chapman, and A. Keys, *J. Am. Med. Assn.* **137,** *1569 (1948)*). A similar trend has been observed in natural starvation. Thus S. Lups and C. Francke (*Nederl. tijdschr. geneesk.* **90,** *764 (1946)*) observed mean decrements of 7, 15, and 20 mm.Hg. in groups of patients over 40, 50, and 60 years of age, with prestarvation mean systolic pressures of 133, 146, and 164 mm. Hg. The experimental and clinical data on blood pressure and other aspects of cardiovascular function in semistarvation were summarized in detail by A. Keys *et al.* ("The Biology of Human Starvation," *esp. p. 609, Univ. of Minnesota Press, Minneapolis (1950)*). Weight loss has been advocated repeatedly for treatment of hypertension, both in the obese and in individuals of normal body weight.

Restriction of the intake of protein as a means of lowering the blood pressure, with caloric needs adequately met by nonprotein foods, found advocates on and off over a period of fifty years. Except for cases of hypertension associated with nephritis (T. Addis, "Glomerular Nephritis," *Macmillan, New York (1948)*) there seems to be no convincing demonstration of the efficacy of a low protein diet as a treatment for high blood pressure. J. L. Guerrant, J. K. Scott, and J. E. Wood (*Am. Heart J.* **26,** *232 (1943)*) reported that protein feeding had no effect on blood pressure unless it was accompanied by a gain in weight. The evidence on the direct effect of carbohydrates, fat, vitamins, and several dietary items, such as watermelon seeds and garlic, is essentially negative.

The topic of dietary treatment of hypertension with emphasis on low salt diets was reviewed by S. S. Kahn and F. J. Stare (*Ann. Rev. Med.* **1,** *127 (1950)*), in the broader context of the role played by nutrition in medicine. Experimental evidence, as well as clinical experiences with the diets, was presented and the dangers involved in the use of low salt dietary regimens were

pointed out. The influence of reduction of caloric intake on hypertension was also briefly considered.

The reviews by Chapman and Gibbons (*loc. cit.*) and by Kahn and Stare (*loc. cit.*) are in agreement on all essential points. It may be useful to combine their conclusions, reached upon critical scrutiny of the evidence.

The dietary treatment, including the diets developed by Allen and Kempner, results in a significant decrease in the blood pressure of some hypertensive patients, especially those with more benign forms of the disease. The degree of effectiveness of this treatment is considered to be of the same order as surgical treatment (sympathectomy). It has not been firmly established whether the hypotensive effect of sodium restriction is due to the effects of the sodium or the chloride ion, although the available evidence points to the dominant importance of sodium. Even more important, it is not fully certain whether the restriction of salt intake is capable of lowering the blood pressure independently of changes in the total caloric intake. At the same time, an increase in the salt intake is likely to increase the blood pressure in cases of renal and essential hypertension. Starvation and semistarvation tend to lower blood pressure, both in normotensive and hypertensive individuals. Hypertension occurs with increased frequency in the obese person and weight reduction in the obese hypertensive patients often brings about a decline in the blood pressure, especially in the systolic component. Reduction in the intake of protein or fat (including cholesterol) does not lower the blood pressure in hypertensive patients and no other dietary factor, fed in quantity or withheld, appears to have a hypotensive effect in patients with hypertension.

There are still many problems that require further investigation. A more effective selection of hypertensive patients for dietary treatment, the optimum level of sodium, protein, and total caloric content of the therapeutic diets, and the possible value of salt-depleting agents, *e.g.*, mercurial diuretics, all require attention. Both reviews emphasize the need for a better control of the conditions, including adequate periods of observation prior to therapy and regard for spontaneous variations in blood pressure, effects of hospitalization, and the response to suggestion and psychotherapy. Larger numbers of homogeneous groups of patients and the use of improved criteria of the functional status of the cardiovascular system will allow the drawing of more valid and definite conclusions about the effectiveness of the dietary treatment of hypertension.

OXALATES IN THE PREVENTION OF DENTAL CARIES IN SYRIAN HAMSTERS

Tooth enamel destruction has been demonstrated in rats by substituting acid beverages for drinking water (*Nutrition Reviews* **4,** *17* (*1946*)). Similar effects are produced in rats when weak solutions of citric, lactic, phosphoric, or sulfuric acid are substituted for drinking water. Oxalic acid solutions, however, were not conducive to tooth destruction. Moreover, when oxalic acid or sodium oxalate was added to the food or drink, the enamel destruction by phosphoric or citric acid was greatly decreased or prevented (*Ibid.* **6,** *75* (*1948*)).

It has been presumed that the protective action of soluble oxalates or oxalic acid is the result of a calculus-like deposit of calcium oxalates forming on the tooth which affords protection against acid decalcification of tooth enamel. R. M. Twedt and F. A. Cajori, at the University of Colorado School of Medicine (*J. Nutrition* **40,** *393* (*1950*)), have conductive studies to determine the

extent to which dietary oxalates actually become incorporated into the tooth structure.

These workers selected the Syrian hamster as the experimental animal because of the high susceptibility of this species to dental caries and because of the morphologic similarities between the teeth of hamsters and human beings as suggested by P. H. Keyes and P. P. Dale (*J. Dent. Res.* **23,** *427 (1944)*).

The Colorado workers used 31 animals in 5 litter-mate groups. At the age of 30 days the animals were placed on a cariogenic diet of the following composition: whole wheat flour 20, corn starch 25, confectioner's sugar 20, powdered whole milk 30, and ground dehydrated alfalfa 5 per cent. The five groups were kept on experiment for periods of 61, 125, 128, 142, and 157 days, respectively. The two groups which were on the experiment for 128 and 142 days had their diets supplemented with 0.5 per cent sodium oxalate at the expense of corn starch.

The hamsters on experiment for only 61 days had a low dental caries incidence. A similar low incidence of dental caries was noted in the 12 hamsters in the two groups receiving the diet supplemented with sodium oxalate for 128 and 142 days, respectively. Among the 13 hamsters on experiment for 125 and 157 days, receiving the same diet but without sodium oxalate supplementation, carious lesions were extensive. In these two groups, the teeth of male animals were more susceptible to decay than those of females.

Oxalic acid analyses were made on the pooled tooth crowns of each animal which was on experiment for over 100 days. The teeth of animals in the two groups receiving sodium oxalate contained an average of 11.8 and 10.2 micrograms, respectively, of oxalic acid per milligram of tooth substance. In contrast, the teeth of hamsters in the two groups receiving the same diet but no supplement of sodium oxalate contained only 3.3 and 1.6 micrograms of oxalic acid per milligram of tooth substance.

The protection against caries observed in the animals receiving oxalate was thus associated with increased concentration of oxalate in the tooth substance. Although these workers did not observe any deposits of calcium oxalate on the teeth surfaces in the oxalate-supplemented animals, some of the teeth were noted to have crystals impacted in fossae and in interproximal spaces.

Since it is generally believed that the tooth decay which occurs when experimental animals are fed high sugar diets is due to the tooth-decalcifying action of lactic acid, the Colorado workers recognized the possibility that the oxalate ion may have protected against dental caries by inhibiting the action of acidogenic bacteria in the oral cavity. However, they considered it more likely that the protective action of oxalates in their experiments was associated with the localization of the oxalate ion in the teeth. It was not definitely established, however, whether the increased concentration of oxalic acid in the teeth of oxalate-supplemented animals was a surface deposit or represented an incorporation of oxalate ion into the chemical structure of the teeth.

Workers at the Naval Medical Research Institute demonstrated that the tooth decalcification in rats resulting from the ingestion of dilute acid solutions (citric, lactic, phosphoric, sulfuric) could be prevented with soluble oxalates (R. A. Gortner, C. M. McCay, J. S. Restarski, and C. A. Schlack, *J. Nutrition* **32,** *121 (1946)*). Workers in the same laboratory later reported that they were unable to obtain any protection with potassium oxalate against tooth decay in white rats or cotton rats receiving a cariogenic diet (S. R. Howell, Schlack, B. L. Taylor, and V. J. Berzinskas, *J. Dent. Res.* **27,** *136 (1948)*). The latter workers, however, used concentrations of potassium oxalate in the drinking water at levels of 0.2 per cent, or less, whereas the Colorado workers used a concentration of 0.5 per cent and included it in the dry ingredients of the diet.

Considerably more work is obviously needed before the value of soluble oxalates can be accurately assessed with regard to mechanism of action and extent of protection against tooth decay associated with high sugar diets in experimental animals.

ADRENOCORTICOTROPIC HORMONE AND GASTRIC SECRETORY FUNCTION

Uropepsin is a proteolytic enzyme which is excreted in the urine of human beings and experimental animals. The nature of the enzyme, its source, and the factors controlling its excretion in the urine have been subjects of many earlier reports. However, only recently have methods of assay been evolved which would provide reasonable accuracy of determination. Certain findings seem now to be well established. Like pepsin of the gastric mucosa, uropepsin is active in a highly acid medium, but unlike the former enzyme is stable in weak alkali, a physicochemical characteristic of pepsinogen, the precursor of pepsin (G. R. Bucher, *Gastroenterology* **8,** *627* (*1947*)).

When pepsinogen is administered intravenously to dogs there is an increase in the uropepsin of the urine. This effect is not observed when pepsin is given orally or administered intravenously (I. A. Mirsky, S. Block, S. Osher, and R. H. Broh-Kahn, *J. Clin. Invest.* **27,** *818* (*1948*)). This evidence would indicate that uropepsin is either pepsinogen or a pepsinogen-like substance. The enzyme in the urine is presumably derived from the gastric mucosa, since it is not found after gastrectomy or in patients who have a diminution or absence of gastric juice. Evidence has been presented indicating that the chief cells of the mucosa of the stomach elaborate uropepsin, releasing the enzyme directly into the circulation for excretion by the kidney (Broh-Kahn, C. J. Podore, and Mirsky, *Ibid.* **27,** *825* (*1948*)).

In normal human subjects the daily rate of excretion of uropepsin is found to be relatively constant. Patients with peptic ulcer excrete this enzyme in quantities approximately twice that of normal individuals (C. J. Podore, R. H. Broh-Kahn, and I. A. Mirsky, *J. Clin. Invest.* **27,** *834* (*1948*)).

Since peptic ulcer represents a disease phenomenon resulting from the stress of living, and adrenocorticotropic hormone (ACTH) is effective in producing the physiologic reactions of stress, H. M. Spiro, R. W. Reifenstein, and S. J. Gray (*J. Lab. Clin. Med.* **35,** *899* (*1950*)) have investigated the effects of ACTH upon gastric secretory function as determined by the urinary excretion of uropepsin. The method of analysis of uropepsin used depended upon enzymatic splitting of hemoglobin as determined by the release of tyrosine-like substances from the substrate at a pH of 1.5. The unit of enzyme activity was taken as that which would release 0.04 mg. of tyrosine-like substance during thirty minutes incubation at 37°C.

In 50 normal human subjects the twenty-four-hour excretion of uropepsin varied from 1050 to 3100 units. The daily excretion rate in an individual was maintained at a constant level and was independent of urine volume. Administration of ACTH was followed by prompt and marked increase in the quantity of uropepsin excreted in 11 subjects. When 100 mg. of ACTH were administered daily to one normal subject, the activity of the enzyme excreted increased from a control level of 1725 units to 6400 units per twenty-four hours after seven days. There was a gradual return to the original excretion rate after termination of ACTH administration. There was no relation between total uropepsin activity and the volume of urine excreted.

In patients with peptic ulcer the urinary excretion of uropepsin was frequently found to be increased. Administration of ACTH

in quantities of 40 mg. per day resulted in further increase of enzyme excretion. The response was not immediate as was observed in normal subjects but was delayed. In one patient the uropepsin excreted increased from 4200 units per twenty-four hours before treatment to 16,500 units after twenty-two days of ACTH administration. The delay in response may have been the result of the somewhat smaller dosage of the hormone used in the patients with peptic ulcer.

The authors administered ACTH to a number of patients with other disease processes who excreted no uropepsin. One of these patients was demonstrated to have atrophic gastritis and another, cancer of the stomach. Response to 50 mg. of ACTH given daily was prompt and rates of excretion nearly reached normal levels in both patients. However, uropepsin was again absent from the urine after administration of the hormone was discontinued. In contrast, there was no demonstrable response to the administration of ACTH in a patient with pernicious anemia and in another following total gastrectomy. There was no uropepsin activity in the urine either prior to or following ACTH administration in these latter patients.

In an interesting extension of these observations it was found that patients with adrenal insufficiency excreted uropepsin at markedly decreased rates. ACTH failed to stimulate excretion of the enzyme but administration of similar quantities of compound E (cortisone) caused uropepsin excretion to increase threefold or nearly to normal levels.

Earlier investigators had demonstrated that uropepsin was either pepsinogen or an enzyme with very similar physicochemical properties. Spiro and his associates were able to demonstrate that the increase in uropepsin excreted in the urine during administration of ACTH was accompanied by increase in the pepsin content of gastric juice which was quantitatively similar and identical in time relationships with hormone administration. This observation permitted the conclusion that the gastric glands were producing pepsinogen under stimulus of ACTH.

These experiments derive their principal significance in that they establish that certain secretory functions of the gastric mucosa are under hormonal control mediated through the adrenal cortex. It is of interest to note that patients with peptic ulcer are slow to respond to ACTH as determined by uropepsin excretion. This might conceivably indicate that there is some degree of adrenal exhaustion in these patients, the result of chronic stress.

The physiologic significance of uropepsin probably has not been wholly elucidated. The enzyme has, to this date, been demonstrated only in the urine. It is reasonable to assume that it has other and more important roles in the body economy and in protein metabolism which are yet to be discovered.

MASSIVE LIVER NECROSIS INDUCED WITH YEAST DIETS IN RATS

Massive liver necrosis and liver cirrhosis, two distinct forms of liver injury, may result when rats are fed diets low in protein or lipotropic factors. Massive liver necrosis, or acute yellow atrophy, is a condition in which whole liver lobules are involved. The disease is characterized by its acuteness. Animals may be dead a few hours after they appear active and well. The animals that survive develop postnecrotic scarring and nodular hyperplasia in the liver.

The course of liver cirrhosis is usually very different. Heavy prolonged fatty infiltration of the liver is invariably antecedent to production of liver cirrhosis in experimental animals. It so happens that the nutritional factors, deficiency of which lead to either liver cirrhosis or massive necrosis,

often tend to occur together in nature. It is for this reason that much confusion has resulted concerning the true etiology of dietary liver injury.

It is now generally agreed that massive liver necrosis is caused by a deficiency of cystine or methionine, and that another factor, an inadequate supply of tocopherol, will favor production of this condition (*Nutrition Reviews* **7,** *302* (*1949*)). The lipotropic factor, choline, does not prevent massive liver necrosis and may even aggravate it.

Diets deficient in both cirrhosis-preventive and necrosis-preventive factors will produce lesions of both types (*Nutrition Reviews* **3,** *122* (*1945*)).

In recent years massive liver necrosis has been produced experimentally by a number of investigators. H. P. Himsworth and L. E. Glynn (*Clin. Sci.* **5,** *93* (*1944*); *J. Path. Bact.* **56,** *297* (*1944*)) presented evidence for the distinct character of this condition and developed diets for its uniform production in experimental animals.

Yeast protein is relatively low in the sulfur-amino acids, cystine and methionine. Diets containing yeast as a source of protein have been used by several workers to produce this condition. Results with yeast diets, however, have not always been consistent. Thus, workers in Germany (A. Hock and H. Fink, *Z. f. physiol. chem.* **279,** *187* (*1943*); K. Schwarz, *Ibid.* **283,** *186* (*1948*)) and Himsworth and his associates in England (*loc. cit.*) have reported almost 100 per cent incidence of massive liver necrosis in rats fed tocopherol-free rations containing yeast as the sole source of protein. On the other hand, P. György and H. Goldblatt in this country (*J. Exp. Med.* **89,** *245* (*1949*)) observed massive liver necrosis in only 40 to 50 per cent of their animals. They achieved reduction in dietary supply of sulfur-amino acids by use of rations low in either yeast or casein.

Recently, P. György, C. S. Rose, R. M. Tomarelli, and H. Goldblatt (*J. Nutrition* **41,** *265* (*1950*)) have compared American yeast with English yeast of the type used by the British investigators in the production of massive liver necrosis in rats. The diets used consisted of yeast 18, corn starch 79, and salt mixture 3 per cent; 0.5 ml. of peanut oil and 2 drops of cod liver oil were added to 8 g. of the dry mixture, just before feeding. In addition each rat received a daily supplement of vitamins, in micrograms as follows: 20 thiamine, 20 pyridoxine, 25 riboflavin, and 100 calcium pantothenate.

Acute massive liver necrosis was produced only in animals receiving the diet containing the British yeast. Out of 19 rats fed this diet, 16 died during the first one hundred and fifty days of the experiment and only one survived the experimental period of two hundred days. The one survivor had a normal liver, and all 18 that died during the experiment had massive liver necrosis. In contrast, when the diet contained American yeast instead of the British yeast, none of the 14 rats had massive liver necrosis.

The British and American yeasts were analyzed for nitrogen, total sulfur, methionine, cystine, and tocopherol. Neither yeast appeared to contain tocopherol. Both yeasts contained the same amount of methionine (0.79 to 0.80 per cent), but the British yeast contained 0.72 $\pm$ 0.05 per cent cystine as compared to 0.43 $\pm$ 0.06 per cent in the American yeast. The total sulfur content for the American yeast was 0.86 per cent compared to 0.65 for the British yeast. The American yeast also contained a greater amount of total nitrogen than did the British yeast (7.83 and 6.76 per cent, respectively). Based on nitrogen content, the American yeast thus contained about 15 per cent more protein and about 30 per cent more total sulfur than the British yeast, yet the British yeast was found to contain considerably more cystine than the American yeast. The two brands of yeast differed in one other respect: the American yeast was a heat-killed brewers' yeast, whereas the British yeast was a live bakers' yeast. Reference is made by the authors to a personal com-

munication from Himsworth and Lindan in which it was reported that the liver-necrotizing effects of live and heat-killed British yeast were equal.

The superiority of the American yeast over the British yeast was also reflected in body weight gains. For example, average gains over the two hundred-day experimental period on the American yeast diet for 14 rats were 69 g. compared to 21 g. for the 19 rats on the British yeast diet. Supplementing the British yeast diet with 3 mg. of tocopherol per rat daily produced average gains of 51 g., and a supplement of 50 mg. per rat per day of cystine on the same diet produced average gains of 88 g. Both of these supplements also completely protected against massive liver necrosis.

The differences in growth obtained by the American and British yeasts were interpreted by the authors as not offering an adequate explanation for the differences in liver-necrotizing potency. They preferred to consider the possibility of unidentified toxic factors in yeast as being responsible for their liver-necrotizing action, and that the sulfur-amino acids and tocopherol exert their protective effect by counteracting these toxic factors. This would obviously imply a relative lack of toxic factors in American yeast and a relatively high concentration of toxic factors in the British yeast.

Such a speculation appears hazardous, particularly in view of the superiority of the American yeast over the British yeast with respect to total nitrogen. Unfortunately the two yeasts were not fed at levels to supply equal quantities of protein in their respective diets. Such an experiment would appear to be essential before it can actually be established whether the British yeast has a greater liver-necrotizing potency than the American yeast. In this connection, it should be mentioned that H. P. Himsworth, in a thorough review of liver diseases ("The Liver and its Diseases," *Blackwell Scientific Publications, Oxford, England* (*1947*)) points out that failure or success in the experimental production of massive liver necrosis may often be due to minor unsuspected differences in the composition of the diet constituents. At least until more concrete evidence is presented in favor of the hypothesis that toxic factors are involved in the production of massive liver necrosis with low protein diets, our present knowledge would seem to favor the more generally accepted view that this condition is a simple nutritional deficiency of sulfur-amino acids and tocopherol.

VITAMIN SUPPLEMENTS AND PERFORMANCE CAPACITY

During World War II, at a time when top industrial production was essential to the war effort, much interest was shown by government and management alike in the nutrition of the industrial worker. This interest was motivated by the conviction that quantitative (caloric) undernutrition and qualitative malnutrition are incompatible with a high work capacity and work effort. This is a generally acceptable thesis but not a very helpful guide to action. Action must be directed toward definite, concrete goals expressed in quantitative terms and should be based on scientific evidence. At the beginning of the war the needed evidence relevant to questions of industrial nutrition was scanty or, in some areas, simply nonexistent.

One of the important topics of industrial nutrition is the value and desirability of administration of vitamins to workers in industry. This question, together with the relevant evidence provided by research, was considered at some length by the Council on Foods and Nutrition and Council on Industrial Health of the American Medical Association (*Arch. Indust. Hyg. Occup. Med.* **1**, *573* (*1950*)).

The Councils have fully recognized the general significance of the role played by vitamins in human nutrition and the therapeutic importance of vitamin preparations. In the review just cited they were concerned only with indiscriminate administration of vitamins to industrial employees. The precedent for free supplements of nutriments by the management was created through the practice of supplying sodium chloride, in tablet form, to workers in certain industries. This was a rational preventive measure. Men working in hot environments lose, in the sweat, large amounts of sodium and of chloride, electrolytes playing an essential role in maintenance of normal body function. At low levels of sodium chloride in the blood, heat cramps are likely to develop. Adequate salt intake prevents the occurrence of this painful and incapacitating muscular disturbance.

The National Nutrition Conference for Defense, held in Washington, D. C., in the spring of 1941, recommended supplemental feeding, using natural foods of high nutritional qualities, in industries in which the workers' diets did not meet the modern standards of adequacy. Little factual information was then available about workers' diets and nutritional status, and an acute need was felt for obtaining such information by use of rigorously controlled studies. Now, almost a decade later, what is the scientific evidence, as distinguished from testimonials and advertising claims, for the value of administration of vitamins to all employees? The Councils feel that "at the present time there is no body of scientific information on which to base the recommendation that industry as a whole adopt this practice" (*loc. cit., p. 577*).

Before reviewing the experimental evidence in some detail, it may be pointed out at the outset that the amount of research directly relevant to the problem is very limited. Except for older and largely inconclusive work on the effect of vitamin A administration (O. H. Schettler, R. F. Bisbee, and B. H. Goodenough, *J. Indust. Hyg. Toxicol.* **21,** *53 (1939)*), the Councils cited as the main source of evidence the study by A. Keys and A. F. Henschel (*J. Nutrition* **23,** *259 (1942)*) and the investigation on aircraft workers in Southern California, directed by H. Borsook and carried out with the cooperation of E. Alpert, J. W. Dubnoff, G. Keighley, and D. G. Wiehl (*Milbank Memorial Fund Quart.* **20,** *329 (1942)*; **21,** *115 (1943)*; **23,** *113 (1945)*; **24,** *99, 251 (1946)*).

The study by Keys and Henschel was designed to test the hypothesis that a direct relation exists between physical vigor and the intake of certain vitamins, above the levels which might conceivably result in subclinical deficiencies. Soldiers maintained on standard U. S. Army garrison rations served as subjects. In four main experimental series on 26 men, large daily supplements of thiamine (5 to 17 mg.), nicotinamide (100 mg.), calcium pantothenate (20 mg.), riboflavin (10 mg.), pyridoxine (10 to 100 mg.), and ascorbic acid (100 to 200 mg.) were administered over periods of four to six weeks, alternating with equal periods of placebo administration. The men were repeatedly subjected to standardized severe physical exercise on a motor-driven treadmill and circulatory, metabolic, and blood chemical responses to the imposed stress were measured. The specific characteristics studied included pulse rate, heart size, stroke output of heart, oxygen consumption, respiratory quotient, urinary nitrogen and ketone body excretion, and concentration in the blood of lactic acid, sugar, hemoglobin, and ketone bodies. In neither a brief but extremely severe exercise nor in prolonged hard physical work had vitamin supplementation a significant effect on ability to carry on exhausting work or on recovery from exertion. It was concluded that healthy young men expending daily about 4000 calories would not be benefited by a daily supply of more than 1.7 mg. of thiamine, 2.4 mg. of riboflavin, and 70 mg. of ascorbic acid.

The effects of supplements of vitamins of

the B-complex (6 mg. thiamine, 80 mg. nicotinamide, 8 mg. riboflavin) were investigated by E. Simonson, N. Enzer, A. Baer, and R. Braun (*J. Indust. Hyg. Toxicol.* **24,** *83 (1942)*). This study was not noted in the Councils' report. No effect was seen on five different types of muscular work, in agreement with the study of Keys and Henschel, but an increase in the frequency at which a flickering light appears to fuse was observed in 8 of the 12 experimental subjects. These subjects reported increased mental alertness and improvement in the general feeling of well-being. The change was interpreted as an increase in the excitability of the retinocortical system, *i.e.*, as a favorable effect. This finding has been, as far as we are aware, neither confirmed nor challenged. The importance of the claim calls obviously for a reinvestigation. For the discussion of recent studies on the effects of thiamine supplementation on learning see *Nutrition Reviews* **4,** *343 (1946)*; **7,** *220 (1949)*.

Borsook and his co-workers studied over 1000 workers at the Lockheed Aircraft Corporation, at Burbank, California, in 1942. The men were divided at random into two groups, one group receiving supplements given five days a weeks for nine to twelve months (50,000 I.U. of vitamin A, 800 I.U. of vitamin D, 10 mg. of thiamine, 10 mg. of riboflavin, 100 mg. of niacinamide, 250 mg. of ascorbic acid), the other a placebo. The number of the subjects was reduced at the end of the study to about 50 per cent by terminations and transfers. For some purposes a third (the control) group was used, matched to the vitamin and placebo group on the basis of company records.

The appraisal of nutritional and physical status involved a physical examination, with special reference to possible evidence of nutritional diseases, analysis of blood and urine, and selected special examinations (record of the condition of the skin, quantitative determination of vibratory sensitivity at different frequencies, record of corneal vascularity, and biomicroscopic examination of the tongue). The medical and dietary histories were taken. During the period of the study the diets remained essentially unchanged. For the appraisal of occupational fitness, data were obtained on absenteeism (unauthorized, due to illness, authorized), termination of employment (military, nonmilitary), and on personnel evaluation (Merit Review ratings).

Comparison of the data obtained in the terminal and the initial medical history revealed no evidence of a consistent beneficial effect of the vitamin supplements on the incidence or severity of upper respiratory infections and on such symptoms as eye discomfort, gastrointestinal malfunction, cramps, feeling of burning feet and pins-and-needles, and irritability. On direct question as to whether the men derived any benefits from the pills, over 70 per cent of the subjects in the placebo and in the vitamin-supplemented group reported benefits such as fewer or less severe colds, better appetite, general improvement in the feeling of well-being, and less eye discomfort. This fact confirms an oft-repeated experience that nutritional studies, especially when involving subjective estimates, are worthless without appropriate controls.

Despite the relatively large doses of vitamin A, thiamine, riboflavin, niacin, and ascorbic acid administered over a period of nine to twelve months, the vitamin group was no better than the placebo group with respect to conjunctival elevations, corneal vascularity, and neurologic abnormalities. There was a statistically significant difference in the thinning of the conjunctiva, the improvement being observed in 23 per cent of the vitamin-supplemented subjects and in 13 per cent of the placebo subjects. There was some suggestion that the supplemented group was better off with reference to the incidence of follicular hyperkeratosis, present in 68 per cent of the vitamin group and in 83 per cent of the placebo group, and the conditions of the tongue, but no data on initial incidence were available and the possi-

bility that these differences were due to the effects of random sampling could not be excluded.

To the authors the persistence of most of the alleged signs of nutritional deficiency in the vitamin subjects after nine to twelve months of supplementation appeared more important than the slight positive clinical results, established or suggested. They drew the conclusion—which has not received attention commensurate with its importance—that these signs must be considered either irreversible results of preceding nutritional deficiency or non-nutritional in origin, but cannot be regarded as evidence of presently existing dietary deficiency. The possibility, most remote as it appears, of greater improvement after a still more prolonged period of therapy, should perhaps temper the definiteness of such a statement. The hematologic picture, except for a rise in the plasma ascorbic level of the vitamin group, was not altered by the vitamin supplementation.

The principal claim for a beneficial effect of the supplements was based on the observations bearing on indirect indexes of industrial morale and work performance. During the first six months there were no significant differences in the absenteeism rates. However, in the second six months the total absenteeism rates of the vitamin subjects were 3.90 days per 100 working days and 4.79 days for the placebo subjects. The difference of 0.89 day was judged to be statistically significant. The difference between the two groups was largest for the unauthorized absences, accounting for 45 per cent of the total difference. The difference in the rates of absenteeism due to illness (2.19 days for the placebo group, 1.98 days per 100 days for the vitamin groups) was not statistically significant. Nonmilitary terminations of employment were somewhat higher in the placebo group than in the vitamin group (13.5 and 8.4 per 100 employees) but the difference did not reach the 5 per cent level of significance. In addition to absenteeism rates and nonmilitary terminations, the results of the Merit Review ratings were utilized in evaluating the effects of the vitamin supplements. Each man was rated by his immediate superior on six characteristics, the rating scale for each characteristic ranging from 1 to 8. The average over-all Merit Review score at the end of the study was 6.22 for the placebo group and 6.38 for the vitamin group; the difference was very small but was considered as highly significant statistically.

The fact that all three indicators of industrial morale—absenteeism rates from causes other than illness, turnover rates, and Merit Review ratings—point in the same direction serves to increase one's confidence in the superiority, marginal though it is, of the vitamin group in the later months of the study. However, there is need for caution. For example, the difference in terminations for nonmilitary reasons was present already at the start of the experiment, with 14 men of the placebo group and 8 men of the vitamin group leaving in March 1942; this was the largest single difference, except for September 1942. Thus differences between the two samples might have been present from the beginning. The ratings of quality and quantity of production, adaptability, knowledge of the job, dependability, and attitude entering into the Merit Review score cannot be considered as truly independent criteria because of the operation of the over-all "halo" effect coloring the rater's judgment of separate characteristics. No actual data on output were provided.

Having in view the total picture, the Councils considered the evidence concerning the value of supplementing "normal" diets by vitamin supplements as lacking or, at the most, tenuous. In addition, the Councils pointed to several positive dangers inherent in indiscriminate vitamin supplementation. It may detract from the fact that many other nutritional factors are needed for adequate nutrition of industrial workers, in addition to vitamins. First of all, the caloric needs must be met by adequate intake of

energy-producing foods. Furthermore, the conviction was expressed that if wholesome natural foods are used as the source of calories, with the caloric intake varying in proportion to the amount of physical work, the required vitamins (and minerals) would likely be secured automatically. The use of supplements of ascorbic acid as a prophylactic against several industrial poisons was considered by the Councils as being still in the experimental stage. It should not be allowed to create a false sense of security on the part of the employer and the employee, which is likely to lead to a lax application of other established safety measures. Also, it should not prevent exploration of other dietary factors, particularly proteins and their constituent amino acids, which might be useful in preventing and treating some types of industrial poisoning. Thus the Council on Pharmacy and Chemistry of the American Medical Association (*J. Am. Med. Assn.* **133,** *107 (1947)*) reported favorably on the value of methionine, one of the amino acids, in the prevention and treatment of liver injuries due to absorbed toxic halogenated hydrocarbons. Another aspect of industrial nutrition which should not be neglected and is not covered by the administration of vitamins is the between-meals feeding.

The impetus to the California study on aircraft workers came from the call for maximum production during the war. It is a telling illustration of the difficulties encountered in industrial studies that output, as noted earlier, has not been included among the measures used in the study. Under unusually favorable circumstances it is feasible to carry out limited studies on nutrition and performance directly in industry. However, rigorous, systematic investigations in the actual factory situations, where the production schedules must be maintained and the quality of work cannot deviate from established standards, is not feasible. Industrial output is the resultant of a complex and variable matrix of factors which are not readily accessible for experimental analysis.

In the study by Keys and Henschel the physical work consisted principally in marching for up to two hours on a motor-driven treadmill at a speed of 3.85 miles per hour and an angle of climb of 10 to 12.5 per cent. The men carried standard packs. This represented hard work and was an appropriate test for the purpose of the study, *i.e.*, for testing whether a superabundance of vitamins is helpful in meeting a physical strain. This type of work together with bicycle riding, another kind of exercise classically studied in the physiologic laboratories, has little or no similarity to the majority of industrial operations. The extrapolation of the results into industrial situations and inferences as to the effects on industrial output are hazardous. Yet output is the touchstone, the final criterion of occupational fitness and, indirectly, of the nutritional status to be improved by vitamin supplementation.

For this reason J. Brožek and J. V. Monke (*Arch. Indust. Hyg. Occup. Med.* **2,** *63 (1950)*) recommended, in a report to the subcommittee on Physiological Stress in Industry (Committee on Nutrition of Industrial Workers, National Research Council) to utilize in studies on nutrition and work capacity the technic of work-miniature situations. This approach had been applied to the evaluation of occupational fitness, industrial training, study of the reciprocal adaptation between men and machines, and research on the effects of physical environment. The miniature-work situations represent a research tool which bridges the gap between the abstract laboratory studies on fitness and performance capacity and the industrial studies. The development and use of laboratory work tests which are in fact "miniatures" of complex industrial jobs would combine the realism of investigations carried out in industrial plants with the systematic, experimental approach characteristic of laboratory research.

J. Brožek, E. Simonson, and A. Keys (*Am. J. Psychol.* **63,** *51 (1950)*) noted in their study on the effects of visual strain that the average level of performance in a standardized visual work test and the decrement in performance taking place in the course of work were more sensitive criteria of the "stress" imposed on the visual system than a large number of analytical tests of visual functions. The same conclusion was reached by N. H. Mackworth (*Med. Res. Council, London, Special Report Series* **268** *(1950)*) on the basis of extensive researches on a wide variety of long-continued operations, such as watching for planes or submarines and working under difficult environmental conditions of extreme heat and humidity. In these investigations the direct measurement of the work output was a better indicator of the changes in fitness than any single physiologic measure.

Ample clinical experience and convincing experimental evidence are at hand that a deficiency of vitamins, particularly those of the B-complex, results in a marked deterioration of the ability to do work. It is hardly surprising, particularly during periods of emergency calling for maximal work effort, that blanket administration of vitamin supplements would be considered and introduced as a sort of "nutritional insurance" and as a potential morale-building measure. It is during peacetime that the merit of such a procedure should be evaluated. Further realistic, rigorously controlled work on the relationship between the intake levels of the critical vitamins and industrial performance is badly needed.

UTILIZATION OF PHYTIN BY CHICKS

The cereal grains, soybeans, and other plant materials contain considerable quantities of phosphorus in the form of phytic acid or its salts. The latter are collectively known as phytin. The phosphorus in phytic acid is bound to the 6-carbons in the cyclic alcohol, inositol, and thus exists in organically bound form.

To what extent phytin phosphorus is utilized is of considerable importance in poultry feeding where the phosphorus contained in cereal grains or soybeans often serves as the chief source of this element in the diet.

M. B. Gillis, L. C. Norris, and G. F. Heuser of Cornell University (*Poultry Sci.* **28,** *283 (1949)*) have determined the extent to which phytin can be relied upon to meet the chicks' need for phosphorus. In one experiment these workers determined the requirement for nonphytin phosphorus in day-old White Leghorn chicks receiving a purified diet containing 0.6 per cent total phosphorus and 1.0 per cent total calcium.

The diet was modified with respect to the source of phosphorus so that data were obtained with the following proportions of inorganic and phytin phosphorus in percentage of the diet: 0.6 inorganic and 0.0 phytin, 0.4 inorganic and 0.2 phytin, 0.2 inorganic and 0.4 phytin, and 0.05 inorganic and 0.55 phytin. The phytin used was a relatively pure calcium phytate.

In another experiment these workers compared varying proportions of inorganic and phytin phosphorus in diets composed of natural foodstuffs. The diets were so compounded that the phytin phosphorus content from natural sources varied from 0.0 to 0.35 per cent of the diet and the total phosphorus and calcium contents were 0.6 and 1.0 per cent, respectively.

Both growth and bone calcification were greatly depressed when two-thirds of the phosphorus in the purified diet was present as phytin. Nearly all of the 15 chicks in this group developed rickets, even in the presence of as much as 160 units of vitamin D_3 per

100 g. of diet. Bone ash values at 4 weeks ranged from 30.6 to 37.8 per cent in the chicks receiving the diet which contained 0.4 per cent phytin phosphorus and 0.2 per cent inorganic phosphorus. In general, the higher bone ash values were found in the chicks receiving high levels of vitamin D. In contrast, when the diet contained 0.4 per cent inorganic phosphorus and 0.2 per cent phytin phosphorus, bone ash values ranged from 34.3 to 46.1 per cent, depending upon the vitamin D level in the diet. When phytin furnished 0.55 per cent of the total of 0.60 per cent phosphorus in the diet, and when the vitamin D content of the diet was below 80 units per 100 g., rickets developed in two weeks, and all chicks died of phosphorus deficiency. Progressively higher levels of vitamin D benefited survival to some extent. The bone ash values of survivors, however, were still considerably below normal (27.5 to 30.1 per cent).

Similar results were obtained in the studies in which natural foodstuffs served as the source of phytin phosphorus. Growth and bone calcification were lowered considerably when the diet contained 0.35 per cent phytin phosphorus and 0.25 per cent inorganic phosphorus, whereas growth and bone calcification were normal when the diet contained 0.2 per cent phytin phosphorus and 0.4 per cent inorganic phosphorus. The poor growth and subnormal bone calcification which resulted with the diet containing 0.35 per cent phytin phosphorus could be corrected by increasing the inorganic phosphorus content from 0.25 per cent to 0.40 per cent, demonstrating that natural diets high in phytin can be corrected simply by supplying readily available forms of phosphorus. The results also emphasized that inorganic phosphorus supplements to a diet high in phytin phosphorus are a much more effective means of correcting the phosphorus deficiency than high levels of vitamin D.

These results confirm those reported earlier by these same workers (M. B. Gillis, L. C. Norris, and G. F. Heuser, *J. Nutrition* **35,** *195 (1948)*). At that time they reported that calcium phytate was almost completely unavailable for chicks receiving diets containing as much as 50 units of pure vitamin D_3 per 100 g. These earlier results emphasized that young chicks require an immediate source of available phosphorus for maintenance of vital functions. Severe phosphorus deficiency resulted in early loss of appetite, weakness, and death in a period of ten to twelve days.

In an earlier review of phytin utilization (*Nutrition Reviews* **6,** *202 (1948)*), the possible role of vitamin D was discussed and it was emphasized that in the presence of adequate vitamin D in the rat, calcium phytate phosphorus was almost as effectively utilized as inorganic phosphorus. From the presently reported study by the Cornell workers, it would appear that the chick differs from the rat in that it is unable to utilize phytin phosphorus effectively even in the presence of high levels of vitamin D.

The results reported by the Cornell investigators challenge the recommended dietary phosphorus allowance for chicks as established in 1944 by the Poultry Subcommittee of the National Research Council Committee on Animal Nutrition. The recommended allowance by the Subcommittee is 0.6 per cent total phosphorus in the diet for growing chicks with the stipulation that one-third of the amount, or 0.2 per cent, must be inorganic phosphorus. The Cornell workers concluded that approximately two-thirds of the 0.6 per cent of phosphorus in the diet must be in the readily available inorganic form for optimum early growth and normal bone calcification.

NOTES

"Doca" and Ascorbic Acid in Arthritis

There has been widespread interest in the combined use of desoxycorticosterone acetate ("doca") and ascorbic acid in the treatment of rheumatoid arthritis since the original report of E. Lewin and E. Wassén (*Lancet* **II,** *993 (1949)*). The possibility that a vitamin-hormone combination, readily available at a reasonable cost, might approach the effect of the rare and costly cortisone or adrenocorticotropic hormone, deserves careful attention indeed. The original report and several subsequent publications, which have both confirmed and denied the original report, have been discussed (*Nutrition Reviews* **8,** *276 (1950)*).

A report by D. H. Kling (*J. Am. Med. Assn.* **143,** *791 (1950)*) is of interest in this connection, since he not only failed to find any beneficial action of this treatment, but observed dangerous side reactions. Fourteen patients with rheumatoid arthritis were given from one to fourteen injections of 5 mg. of "doca" followed in a few minutes by 1000 mg. of ascorbic acid intravenously or intramuscularly. Two patients received daily injections and the rest were given injections two or three times weekly. Salt intake was restricted. Only one patient showed objective improvement and one had subjective improvement only. Six patients were not improved and 6 became worse; 5 of these patients had acute exacerbations. Sudden large effusions of both knee joints developed in 3 patients. Hypertension developed in 2 patients, complicated by heart failure in one. One patient showed pronounced muscular weakness and soreness associated with a low serum potassium level. Accordingly, Kling concludes that these dangerous reactions make this treatment too hazardous to use, even if striking improvement were produced in every case.

The reason for the high incidence of toxic reactions in Kling's patients and the lack of notable untoward effects in previous studies are not evident. However, in view of the fact that "doca" is known to be capable of producing undesirable reactions in both man and animals under certain conditions, it would seem wise to approach the use of it in arthritis with caution, until the treatment is on a sounder basis and a calm and critical appraisal of its potential dangers is made.

A New Pancreatic Hormone

Two articles by H. De Waele (*Arch. internat. de physiol.* **55,** *209 (1948); 57, 133 (1949)*) may open a new chapter in the history of our knowledge of the endocrine function of the pancreas. In these articles the author describes his demonstration that the pancreas secretes a metabolic hormone other than insulin (this hormone he calls, somewhat improperly perhaps, pancreatic anti-insulin), his isolation and characterization of the hormone, and his analysis of its metabolic effects on carbohydrates and lipids. The method of preparation of the new hormone is different from that followed by L. R. Dragstedt *et al.* (*Am. J. Physiol.* **127,** *755 (1939)*) to prepare "lipocaic" and its properties are distinct from Dragstedt's extract.

The question which De Waele was trying to solve was whether the pancreas secretes a hormone which might mobilize carbohydrates and lipids either in the liver or in the periphery or both. The starting materials for the hormone preparation were beef and pork pancreas. The novel method of attack was the method of fractionation, namely, the simultaneous use of salt solutions and fat solvents. In later modifications, other biphasic systems at various temperatures were used. The yield of material from pancreas is of the same order as that for insulin.

The hormone can be used in very large doses by mouth (2 g.) or by injections of small doses (less than 1 mg.) intravenously. Its primary effects, as studied in dogs, rabbits, rats, mice, and frogs are as follows: it produces hyperglycemia and glucosuria; it causes an increase in the resting metabolic rate; it decreases the glycogen content of both liver and muscles; it inhibits the glycogen deposition when glucose is injected simultaneously with the hormone; concomitantly with the decrease of glycogen in liver and muscles it causes an increase in the peripheral and muscle fat and protects the liver against excessive fat deposition; it acts (and this includes the increase in metabolic rate) in the absence of the thyroid gland; it acts in the absence of the pancreas; it acts in the absence of the adrenals and in animals with cervical sections of the vagi; it has a parasympatheticomimetic action as shown by its effects on the blood sugar and on the contractions of the intestine of the rabbit.

The author was able to devise a biologic method for the quantitative bioassay of the hormone, based on the inhibition of glycogen deposition in the frog injected simultaneously with glucose. It was this test which enabled him to concentrate successfully the hormone, crystallize it, and estimate the normal content in the pancreas. Furthermore, he was able to detect a substance with identical action in diabetic urine and, in smaller amounts, in normal urine.

If these findings are confirmed, our understanding of the normal (and pathologic) regulations of carbohydrate and lipid metabolism will have to be revised. Meanwhile it may be remarked that rarely has such a thorough study of a new physiologic phenomenon been thus presented as a first publication.

Determination of the Mass of Single Cells

Among recent developments in biologic technics few present more exciting possibilities than the new quantitative microtechnics. A new method (A. Engström and B. Lindström, *Biochim. et biophys. acta* **4**, *351 (1950)*) opens the possibility of weighing single cells, *i.e.*, of putting cytochemistry on a solid quantitative basis.

The method is based on the well-known property of biologic material of absorbing x-rays. This absorption is compared with that given by suitable standards. In practice, a continuous spectrum of x-rays is filtered through a proper aluminum foil filter. The beam is then directed either through the biologic sample or through the standard, a step wedge of thin-absorbing foils of nitrocellulose. The transmitted beam is received on the photographic emulsion of a Lippmann film. The intensities of the transmitted beams are compared by photometric measurements on the enlarged negative.

To derive from these data the mass of the sample, certain postulates have to be made. For instance, as a first approximation, the biologic material is assumed to be built up of only carbon, nitrogen, and oxygen. This is justifiable if one considers that all other elements, except hydrogen, are found in relatively minute concentrations. Hydrogen itself causes only small absorption and can be accounted for by a separate correction, the detail of which is given. The calculation of the mass of the sample, once these postulates are accepted and experimental data obtained, presents no difficulty.

This new development will permit nutritionists to carry to the level of the single cell the criterion of weight so constantly used for the entire animal. It will no doubt prove invaluable in a variety of problems of growth, including normal, abnormal, and aging.